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Doctor of Philosophy Degree
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ABSTRACT

Title of Thesis: Effects of Repeated Acute Stress in Obese and Non-obese Rats

Christie O. Simpson-McKenzie, Doctor of Philosophy, 2008

Thesis directed by: Neil E. Grunberg, Ph.D.

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Stress is pervasive among all people. Roughly 65% of American adults are overweight or obese. Because stress and excess body weight each has deleterious mental and physical health effects, it is relevant to determine whether the two conditions exacerbate each others' effects.

This doctoral research project examined responses to stress in male and female, lean and obese rats. Two separate experiments examined behavioral and biological effects of repeated acute stress as a function of body weight, diet, and sex. Experiment I manipulated genetic and environmental variables to examine effects of repeated acute stress on obese and non-obese male rats. Experiment II examined effects of repeated acute stress on genetically obese and non-obese male and female rats.

In Experiment I, stress: (1) decreased bland food consumption in lean and obese rats; (2) altered cafeteria food consumption; (3) reduced caloric consumption in most groups; (4) increased blood corticosterone levels, especially among lean rats re-exposed to stress; (5) increased physical activity among lean rats; and (6) increased startle responses among lean rats but impaired attentional processing among animals fed cafeteria food.

In Experiment II, subsequent to stress (i.e., post-stress): (1) prior stress exposure resulted in lower corticosterone levels compared to a no-stress history, especially among obese rats; (2) startle responses among lean males increased; (3) percent pre-pulse inhibition of startle increased among lean stressed females but decreased among obese stressed females.

Together these findings indicate that obese rats were less reactive to stress. The interpretation of these findings is that obesity is associated with maladaptive stress responses.

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This work is dedicated to my parents, Fred (late) and Barbara Oates

who modeled compassion, diligence, and integrity.

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Effects of Repeated Acute Stress in Obese and Non-obese Rats

by

Christie O. Simpson-McKenzie

Dissertation submitted to the faculty of the

Department of Medical and Clinical Psychology

Graduate Program of the

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in partial fulfillment

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SECTION I: INTRODUCTION

OVERVIEW

Stress is pervasive in the lives of many people, including individuals in life-threatening jobs (e.g., military troops, law enforcement officers, firefighters) and individuals who face social and psychological shunning and bias (e.g., obese individuals and some members of minority groups) (Fiscella & Franks, 1997; Swinburn & Egger, 2004). Behavioral, cognitive, and biological changes are important to manage the stressful event or eliminate the consequences of stress. The failure to change behavioral, cognitive, and biological responses when faced with a stressor that demands adaptation is maladaptive and may result in negative long-term consequences. When responses to stress are maladaptive, stress can result in negative mental and physical effects, including anxiety disorders, depression, cardiovascular diseases, diabetes, and immune disorders (Baum, Gatchel, & Krantz, 1997; McEwen, 1998b). In fact, stress has been implicated in 40% of poor health outcomes (Phillips, Kiernan, & King, 2001).

Excessive body weight affects many people, with roughly 65% of American adults either overweight or obese (Flegal, Carroll, Ogden, & Johnson, 2002). Excessive body weight results in increased risk of premature mortality (over 112,000 deaths each year in the U.S.), cardiovascular diseases, diabetes, and some cancers (Flegal, Graubard, Williamson, & Gail, 2005; Flegal, Williamson, Pamuk, & Rosenberg, 2004). Excessive body weight is reaching epidemic proportions in the U.S. as the incidence of obesity has increased one-third over the past decade (Friedman, 2003).

With stress and excessive body weight so commonplace and with each condition causing deleterious mental and physical health effects, it is relevant to determine whether the two conditions exacerbate each others' effects. Simply put, are maladaptive stress responses greater in individuals with excessive body weight? Further, because there are gender differences in stress responses and in overweight and obesity prevalence, do stress responses in obese and non-obese individuals differ based on sex (male vs. female)? These questions were the focus of this doctoral dissertation research.

The U.S. military is experiencing an obesity trend that mirrors the general population. Approximately 54% of military personnel are overweight (Lindquist & Bray, 2001) and 13% meet criteria for obesity (NQMP, 2003). It is noteworthy that the increase in body weight among military personnel is not explained by a decrease in physical activity. Unlike the general population in which 60% of adults do not engage in regular physical activity (USDHHS, 1996), about 67% of military personnel report engaging in vigorous physical activity on three or more days per week (Lindquist & Bray, 2001). The growing problem of overweight and obesity among military personnel negatively affects performance and operational readiness.

In addition to the negative effects of overweight and obesity among military personnel, there is the danger of stress because stress levels are high among military personnel. A recent Department of Defense survey reported that about one-third of military personnel report high amounts of work-related stress (e.g., separation from family, deployment, amount of work) (Bray et al., 2003). About 28% of military personnel surveyed reported that work or family stressors adversely

impacted their perceived job performance (Bray et al., 2003). Those personnel characterized by high stress were twice as likely as moderate or low stress groups to have four or more days of lost productivity as demonstrated by working below normal performance level, arriving to work late by 30 minutes or more, attributing work absence to poor health, or injuring themselves at the worksite in the past 12 months (Bray et al., 2003). If stress responses increase with body weight, then the combined effects of high stress and high prevalence of excessive body weight may be particularly damaging to health, operational readiness, and performance.

The present work used an animal model to investigate whether stress responses differ between obese and non-obese individuals and, in particular, whether responses to stress in obese individuals are maladaptive compared to stress responses in lean individuals. This approach allowed for the manipulation of stress, the investigation of genetic and environmental factors, daily measurement over several weeks, and control of variables in a true experiment.

The present research included two experiments. Experiment I examined behavioral and biological effects of stress in genetically obese (fatty Zucker) and non-obese (Sprague-Dawley) male rats with access to either standard chow or high-caloric foods and standard chow. Experiment II built upon the findings of Experiment I by examining the effects of stress in male and female genetically obese (fatty Zuckers) and non-obese (lean Zuckers) rats. In these two experiments: (1) genetically obese and non-obese rats served as subjects; (2) diet was controlled to compare diet-induced excessive body weight and genetically-bred excessive body weight; (3) housing was controlled to examine environmental influences; and (4)

stress exposure was manipulated. The dependent variables included: body weight, food consumption, physical activity, behavioral indices of attention and anxiety, and a biochemical measure of the stress response.

Section I reviews background material relevant to the proposed research, including a history of the stress concept, impact of stress on physical and mental health, burden of obesity, and the relationship among stress, feeding, and body weight. Section II presents the experimental design, measures, and procedures for Experiments I and II. Section III provides a summary and discussion of the findings for this project. Areas for future studies also are included in this section. Section IV includes Tables, Figures, and References.

BACKGROUND

STRESS

Definition. Stress is a process in which biological, psychological, and environmental events called stressors challenge an organism (Cannon, 1929; Baum et al., 1997). The adaptation or coping in the face of real, implied, or perceived threat is the stress response. How organisms cope may effectively neutralize the stressors (i.e., adaptive responses) or may set the stage for illness and disease. Among researchers and health professionals, stress is characterized by type and duration.

Stress gives rise to a pattern of arousal that increases the body's general state of readiness and alertness. Note that physiological stress responses are heavily biased toward maintaining energy availability via the release of glucocorticoids. If excess glucose remains in the bloodstream once energy demands are satisfied, then insulin promotes storage of the excess energy as fat (Guyton & Hall, 2000). Therefore, those individuals who fail to expend the energy that is mobilized to manage the physical demands of stress may be prone to gain excess body weight and subsequently to develop other negative health consequences. Whether obese male and female individuals mobilize and expend energy in response to stress differently from lean individuals is unclear.

Types. Physical stressors are those stimuli that have a direct threat on survival (Lovallo, 1997). Examples of physical stressors include extreme temperatures, infection, and toxic substances. Other stimuli that do not present a direct physical threat are psychological stressors. This type of stressor includes

one's interpretations about certain stimuli that are symbols of threat, harm, or loss (Baum et al., 1997).

Duration. Acute stress results from transient stressors (APA, 2006). These kinds of stressors occur in small doses. Acute stress is associated with immediate responses to perceived threats on the body. In quick bursts, acute stress can optimize performance by activating the sympathetic adrenomedullary (SAM) response, which essentially is the bodily alarm system (McEwen, 1998a). This "fight or flight" response is meant to be an adaptation mechanism for brief periods of stress. Although protective in most instances, acute stress has been linked to cardiovascular diseases (Baum et al., 1997). Repeated stress occurs episodically for brief periods of time. When sufficient time to recover is not allotted before the onset of the next stressor or the stress response is activated too often, the net effect is "wear and tear" on the body. These short, but frequent, bouts of stress can have the same negative long-term consequences on the body as seen in chronic stress conditions (McEwen, 1998a, 1998b). Chronic stress is a state of persistent physiological arousal over long periods of time. Under chronic stress conditions, the body sustains a heightened level of physiological arousal, which exerts damage on the whole body. The prolonged effects of stress are associated with gastrointestinal, cardiovascular, immune, respiratory, metabolic, and psychological disorders (McEwen, 1998b).

History of Stress Concept

Modern understanding of stress incorporates biology, psychology, and social factors. The relationship between stress and disease has been recognized for more than a century. Historically, these factors emerged from separate traditions.

Cannon. In 1935, Walter Cannon coined the term *homeostasis* to describe steady states maintained by different bodily systems to protect the organism from external influences. He described a well-orchestrated process in which the sympathetic nervous system communicates with the adrenal glands to produce physiologic responses that maintain steady states via the actions of adrenaline. Regardless of the stressful stimulus — extreme temperatures, low oxygen pressure, hemorrhage, hypoglycemia — Cannon found that the sympathetic adrenomedullary mechanism (SAM) is activated in reaction to the stimulus and its primary function is to preserve the body's steady state (Cannon, 1935). Cannon suggested that when the body becomes overloaded with multiple unstable conditions, the cumulative strain causes the sympathetic adrenomedullary system to decompensate, rendering the system ineffective to restore balance to the body. It is at this point that physical and psychological disease states appear. Cannon speculated that all organisms have a stress threshold and limits to the conditions in which their body can function effectively (Cannon, 1935). Cannon also observed that because SAM activation prepares the body for vigorous physical exertion, adaptive responses to acute stressors include energy-consuming physical action. The failure to expend the mobilized energy sources, Cannon believed, was the harbinger of stress-related

diseases. To fend off stress in a modern society in which threats generally cannot be physically fought or fled from, Cannon recommended exercise.

Selye. Hans Selye defined stress as a nonspecific response of the body to any demand placed upon it. He proposed that there was one stereotyped response pattern that the body activates in order to adapt to the increased demand regardless of the type of stressor. More specifically, he conceptualized stress as the "General Adaptation Syndrome" with three distinct stages: (1) Alarm, (2) Resistance, (3) Exhaustion. In his early work with rats, Selye observed that noxious stimuli or physical stressors, such as extreme temperatures and toxic agents, could not be tolerated indefinitely. The alarm reaction referred to the initial and immediate response made by the body in an attempt to adapt to the stressor. Reducing or otherwise adapting to the stressor constituted the stage of resistance. Because adaptation to severe and prolonged stressors expends energy, over time bodily resources are depleted and the last stage of exhaustion (and ultimately death) ensues. This process, Selye theorized, was orchestrated by the hypothalamic-pituitary-adrenocortical (HPA) axis. He described a sequence of events in response to an acute stressor in which the hypothalamus signals the pituitary gland (via corticotrophin-releasing factor), the pituitary gland in turn releases adrenocorticotrophic hormone (ACTH), and the adrenal gland produces cortisol (in humans) and corticosterone (in rodents). Like Cannon, Selye believed that constant exposure to stress is harmful to the organism. He further proposed that dysregulation of the general adaptation syndrome (or the HPA axis) led to "diseases

of adaptation" such as emotional irregularities, headaches, insomnia, upset stomach, and hypertension (Selye, 1973).

Mason. John Mason emphasized that psychological variables powerfully affect physiological responses, including HPA axis responses and sympathetic responses (Mason, 1971, 1975a, 1975b). Mason suggested that psychological variables may mediate the stress response (Mason, 1971, 1975a, 1975b).

Lazarus and other psychosocial theorists. Richard Lazarus expanded on Mason's position that psychological variables may influence an individual's response to stress. Lazarus introduced the concept of primary and secondary appraisal in which an individual subjectively evaluates a stressor as a positive, negative, or neutral stimulus and then employs a coping response in order to deal with the stressor and/or manage the emotional responses provoked by the stressor (Cohen, Keesler, & Gordon, 1995). Appraisal is shaped by mood, personality, and/or psychopathology.

Other seminal studies supported the influence of psychosocial variables on the stress response. Glass and Singer (1972) demonstrated the importance of control and predictability such that individuals who had perceived control over a loud, predictable noise readily adapt to this aversive stimulus. Lack of perceived control in the work environment increased the release of catecholamines (Frankenhaeuser & Gardell, 1976). Richard Rahe focused on the cumulative amount of change related to significant life events, concluding that the number of life changes was positively correlated with an individual's susceptibility to develop

psychological and physical disease states (Holmes & Rahe, 1967; Rahe & Arthur, 1978).

McEwen. Bruce McEwen proposed that "allostasis" augments homeostasis by changing hormonal set points and other controls that are in place to maintain an organism's response to stress (McEwen & Wingfield, 2003). McEwen conceptualized maladaptive responses to stress as an "allostatic load" in which the stress response has been overactivated and higher than normal amounts of glucocorticoids are secreted (McEwen, 1998b; McEwen & Wingfield, 2003). McEwen describes four conditions in which an allostatic load may occur: (1) frequent exposure to stressful conditions, (2) prolonged exposure to stressful conditions can lead to a series of maladaptive processes that are mediated by individual differences, perceived stress, as well as by behavioral and physiological responses; (3) specific body systems lose their ability to regulate themselves, causing compensatory sequelae across other body systems, which effectively activates the stress response inappropriately; and (4) the cross-talk between the sympathetic nervous system and the HPA axis may become dysregulated, altering the set point for stress hormone levels in the body. The effects of a dysregulated stress responses and, in particular, elevated cortisol levels are evident in the brain and periphery, affecting the immune, cardiovascular, and metabolic systems.

Effects of Stress

In human and rodents, stress can result in negative emotions, behavioral disruptions, and physiological reactions (Baum et al., 1997; Baum, Grunberg, &

Singer, 1982). This section emphasizes animal research because the present research used rats as subjects.

Behavioral Effects of Stress. Animal studies have established that behavioral responses to stress include impairments in learning and memory, an increase in behavioral indices of anxiety and depression, and changes in feeding, body weight, and pain tolerance. Learning and memory disturbances, low energy, and negative mood are common responses to chronic stress (McEwen, 1998a). Food preferences in humans also may change in response to repeated stress such that weight-promoting foods (i.e., high-fat and high-carbohydrate food stuffs) tend to be consumed by some people more during stressful periods than do bland foods (Greeno & Wing, 1994; Grunberg & Straub, 1992; Pecoraro, Reyes, Gomez, Bhargava, & Dallman, 2004). Comfort foods, particularly those high in carbohydrates, may trigger a cascade of biochemical reactions that ultimately increase the mood-enhancing neurotransmitter serotonin (Wurtman & Wurtman, 1995). One explanation is that elevated corticosteroid levels lead to the consumption of carbohydrate-dense foods that, in turn, attenuates the negative effects of stress on mood. High levels of corticosteroids have been associated with increased carbohydrate craving (Epel, Lapidus, McEwen, & Brownell, 2001). Some animal studies report that consumption of high-energy foods increases (Ely et al., 1997; Levin et al., 2000) and consumption of bland chow increases (Levine & Morley, 1981) during stressful periods. The present research included the examination of whether high-energy foods reduce the stress response.

Biological Effects of Stress. There are two primary systems responsible for the physiological response to stress: (1) sympathetic adrenomedullary (SAM) system and (2) the hypothalamic-pituitary-adrenal (HPA) axis. The SAM system is initially activated in response to stress. Sympathetic nerve fibers innervate the adrenal medulla to release catecholamines (e.g., epinephrine and norepinephrine) into the bloodstream (Baum et al., 1982; Everly & Lating, 2002). The HPA axis secretes glucocorticoids that sustain the physiological arousal (Everly & Lating, 2002). Glucocorticoids are essential to ensure relatively stable levels of circulating glucose during normal cycles of feeding, resting, and activity – events that vary in terms of energy demand and expenditure. Cortisol is also critical in the stressed state to maintain energy availability and to allow the individual to resist and to cope with physical, metabolic, and psychological stressors (Guyton & Hall, 2000). In the presence of an appropriately functioning HPA axis, cortisol responses are tightly regulated and quickly diminish with cessation of or adaptation to a stressful experience (Guyton & Hall, 2000). When adaptive coping occurs, the peak level of corticosterone occurs approximately 30 minutes after the stressor terminates (Garcia, Marti, Valles, Dal-Zotto, & Armario, 2000). Some studies have reported that basal cortisol levels in obese individuals are less than normal weight controls (Jessop, Dallman, Fleming, & Lightman, 2001; Korbonits et al., 1996). Others have reported that basal cortisol levels are elevated in obese individuals (Pasquali & Vicennati, 2000; Stunkard, Faith, & Allison, 2003). The relationship between elevated cortisol and weight is strongest in individuals with abdominal obesity (Chrousos, 2000). The contradictory findings in the literature suggest that further

investigation is warranted to determine if cortisol levels in rested and stressed states differ depending on body weight.

Stress Recovery Periods

Adaptive responses to stressful stimuli are those that allow the animal to function or to cope in the context of a challenging environment. Glass and Singer (1972) suggested that maladaptive responses do not occur while the stressor is present. Rather, they occur once the stressor has been terminated. *Aftereffects* is the term used to describe this phenomenon (Glass & Singer, 1972). These Post-stress effects are associated with decreased cognitive performance, lowered threshold for frustration, reduced sensitivity toward others, and increased aggression (Baum et al., 1997; Glass & Singer, 1972). Aftereffects may result from the amount of effort expended to manage the stressor. Theoretically, a high amount of effort used to cope with a stressor reduces the reserve capacity to deal with subsequent demands. This effect is particularly marked if recovery periods are interrupted to manage another stressor. In the present work, the aftereffects of stress were examined by including a Post-stress period.

Individual Differences in Stress Responses

Sex. Sex alters the stress response. Women have a higher prevalence of anxiety, depressive, and eating disorders than do men (APA, 2001; NIMH, 2001). If these mental health conditions are, in part, responses to stress, then women may have different reactions to stress than men. Coping styles also differ between the sexes. Females are more likely to seek help from others to attenuate the stress response, which is a pattern referred to as "tend-and-befriend." There is speculation

that the neurochemical bases for this tendency among females are oxytocin and sex-specific hormones (Taylor et al., 2000). On the other hand, males characteristically exhibit the fight or flight response, in which energy is mobilized to attack (Taylor et al., 2000).

In animal laboratory studies, sex differences have been reported in studies of biobehavioral responses to stress. After 21 days of restraint stress, food consumption (bland chow) decreased among male rats, but was not affected among female rats (Faraday, 2002). In another study, after a single exposure to restraint stress, male rats spent less time in the center of an open-field arena (indicating more anxiety) but more time engaged in grooming behaviors than did females (indicating less anxiety) (Albonetti & Farabollini, 1992). The investigators concluded that males displayed more anxiety-like behavior in the open field than did females. However, the excessive grooming among females compared to males can also be interpreted as a displacement behavior that occurs when an animal is having conflict between behavioral drives (e.g., fight or flight). In two separate studies using repeated restraint stress, males exhibited more anxiety-like responses in the elevated plus maze compared to females (Albonetti & Farabollini, 1992; Chadda & Devaud, 2005). In response to 21 days of repeated restraint stress, female rats produced more corticosterone and for a longer period compared to male rats (Galea et al., 1997). Female rats displayed more defensive postures in response to 7 days of predator stress than did male rats (Klein, Lambert, Durr, Schaefer, & Waring, 1994). Females had decreased activity in open field chamber and increased defecation after 24 days of unpredictable chronic stress compared to male rats (Renard, Rivarola, & Suarez,

2007). Male rats had more stress-induced analgesic responses to cold water stress than did females (Romero & Bodnar, 1986). These findings suggest that stress responses differ based on sex.

Body type and feeding behavior. Two hypotheses suggest that eating behavior differs by body type (Greeno & Wing, 1994). Schachter's (1968) internality-externality hypothesis suggests that normal weight individuals primarily eat in response to internal cues associated with hunger (e.g., gastric contractions), whereas obese individuals primarily eat in response to external cues. Gastric contractions are reduced in response to stress (Cannon, 1915; Carlson, 1916). The internality-externality hypothesis predicts that normal weight individuals will not eat in response to stress because the internal cues (e.g., gastric contractions) to eat are reduced during stress. In contrast, this hypothesis predicts that obese individuals will eat in response to external cues, such as stressors in the environment (Schachter, Goldman, & Gordon, 1968). Psychosomatic theory suggests that obese individuals associate stressful states with hunger and are likely to respond by eating (Greeno & Wing, 1994). The implication of these two theoretical assumptions is that obese and normal weight individuals respond differently to stress with regard to eating behavior.

Body type, sex, and stress responses. This doctoral dissertation research was inspired by the investigator's master's thesis. In this master's project, obese and non-obese African-American and Caucasian men and women participated in vigorous exercise and a meal challenge (i.e., maximal treadmill exercise and liquid Ensure™). Responses to exercise and meal challenges differed by ethnicity and

body weight. African Americans and obese individuals had substantially lower cortisol levels compared to non-obese and Caucasian individuals over the entire testing period, suggesting that basal levels and stress-induced changes in HPA axis hormones are influenced by ethnicity and body weight (Oates, 2006). Specifically, African Americans had blunted cortisol responses, which is a pattern similarly observed among obese individuals (Jessop, Dallman, & Lightman, 2001). Because cortisol responses to stressors are intended to fuel the body to deal with the stressor, blunted cortisol responses may be problematic in the face of an environmental demand. The findings of the broader project, of which the master's thesis was a part, have not been completely analyzed with regard to differences in psychological and biological responses of obese and non-obese people to vigorous exercise and meal challenge conditions.

Few animal studies have examined stress responses by body type. In a study of male rats with high amounts of abdominal fat mass, home cage activity was reduced after social defeat (e.g., 1 hour exposure to an aggressive male) (Buwalda, Blom, Koolhaus, & van Dijk, 2001). Michel and colleagues (2003) exposed obese rats and non-obese rats to two episodes of 20-minute restraint stress and fed them different diets (i.e., standard chow or high-energy/medium-fat). In the nine days following the initial exposure to restraint, obese stressed rats that were fed standard chow gained significantly less weight than did obese unstressed rats that were fed standard chow. However, when obese stressed rats were fed a medium-fat diet, they gained more weight than did obese unstressed rats fed the same diet and non-obese rats fed either a high-energy diet or standard chow (Michel et al., 2003). A

high-energy diet increased body weight without an associated increase in food consumption in obese stressed rats, but standard chow decreased body weight in obese stressed rats. The investigators suggested that obese rats were sensitive to diet-induced effects of a single restraint exposure on body weight gain. In response to a second exposure to restraint, obese stressed rats, regardless of diet (e.g., standard chow or high-energy/medium-fat), had greater levels of horizontal activity in an open field chamber than did stressed non-obese rats during the first 7 of 15 minutes (Michel et al., 2003). Note that increased lateral (e.g., horizontal activity) or rearing (e.g., vertical activity) movement in a novel environment is associated with adaptive responses to stress. In the same study, obese stressed rats fed a medium-fat diet had greater levels of vertical activity after 15 minutes in the open field chamber than did non-obese stressed rats (Michel et al., 2003). The investigators concluded that obese stressed rats demonstrated less anxiety-like behavior in the open field because they were more active than non-obese stressed rats. The findings from this study indicate that examining stress responses by body weight warrants further study.

Levin and colleagues (2000) exposed obese rats and non-obese rats fed a high-energy diet to a 5-week chronic, moderate stress paradigm (i.e., random stressors such as 15 minutes of restraint stress, switching home cage with another animal, 10 minutes of exposure to another male in the experimental rat's home cage, 2 minutes of forced swim in room temperature water, and saline injection). Non-obese stressed rats gained less body weight than did obese stressed rats, but there was no decrease in food consumption for either body type. There were no

significant differences between obese and non-obese rats in response to stress in 10 minutes of open field testing. The investigators concluded that non-obese rats were sensitive and obese rats were hyporesponsive to chronic stress. The animal studies reviewed suggest that: (1) physical activity responses to stress may depend on testing environment – open field vs. open cage — and other variables, such as duration and type of stressors; (2) the positive association between body weight and feeding is decoupled among obese stressed rats. Despite eating similar amounts of food, obese stressed rats gained more weight than did lean stressed rats. The weakness of the previous studies that measured open field activity is the short observation period of only 10-15 minutes. Overall physical activity in response to stress cannot be separated from these short observation periods because the animal is adapting to the novel testing environment (Faraday, 2002; Elliott & Grunberg, 2005).

Extrapolating from animal models of cardiovascular disease, there is evidence that obese and non-obese differ in stress responses. For instance, obese Zucker rats had reduced vagal tone in response to stress (i.e., 2-second inescapable footshock) compared to lean Zucker rats (Nyakas, Balkan, Steffens, & Bohus, 1995). In the same study, obese Zucker rats displayed longer Post-stress freezing behavior than did lean Zucker rats but the difference did not reach statistical significance (Nyakas et al., 1995). The prolonged immobility reported among obese Zucker rats may indicate that obese animals require longer periods of time to fully recover from stress.

Neuroendocrine differences in the primary stress hormone, corticosterone, have been reported between obese and non-obese. Obese rats have lower expression of glucocorticoid receptors than do non-obese rats (Michel et al., 2004). Reduced expression of glucocorticoid receptors is associated with repeated or chronic stress (Michel et al., 2004) and with an inability to efficiently turn off the HPA axis once the stressor ceases. Levin and colleagues (2000) manipulated body weight in a substrain of Sprague-Dawley rats bred for high and low weight gain based on an energy-rich diet. These investigators reported that five weeks of random, chronic stress significantly elevated corticosterone levels in non-obese stressed rats but not obese stressed rats. These investigators concluded that non-obese rats are hyperresponsive and obese rats are hyporesponsive to chronic stress.

In contrast, Guillaume-Gentil and colleagues (1990) exposed obese Zucker rats to multiple different stressors (e.g., single 1-hour episode of immobilization, 2-hour periods of cold for 7 days, and five consecutive exposures to 1-minute periods of ether vapor). Obese Zucker rats had higher corticosterone levels up to 3 hours after exposure to the initial stressor (a single 1-hour period of immobilization) and in response to each subsequent stressor compared to lean Zucker rats (Guillaume-Gentil et al., 1990). Furthermore, obese Zucker males produced greater amounts of corticosterone than all other groups (e.g., lean Zucker males and females and obese Zucker females) regardless of stressor. The present experiment used repeated exposures to 20 min restraint stress; it is possible that repeated exposure to the same stressor will elicit a different pattern of responses compared to repeated

exposure to different stressors. Taken together, the discrepant findings across studies may reflect true differences in obese and non-obese rats or methodological differences in how obesity was manipulated (diet vs. genetics) and how stress was operationalized.

Stress Vulnerability and Reactivity

Stress vulnerability refers to the increased likelihood that certain genetic, biological, psychological, social, and environmental factors result in specific disease states or stress-related outcomes (Baum et al., 1997; Faraday, 2000). These factors have strong causal or correlational links with stress-related outcomes and may predispose a particular subgroup of individuals to poorer health outcomes. Of clinical relevance is the fact that these factors may identify vulnerable groups and lead to either prevention of or earlier intervention for stress-related diseases. The construct of vulnerability includes biopsychosocial aspects relevant to the individual: genetics, sex, body type, personality traits, developmental experiences, and environment (Faraday, 2000). These aspects of the individual directly influence the adaptive or coping response employed to reduce or to terminate the stressor.

Stress reactivity refers to the behavioral, cognitive, and biological changes that occur in response to the stressor. In experimental studies, reactivity is quantified as the difference in functioning before the stressor and after the onset of the stressor (Baum et al., 1997). The onset of a stressor results in short-term responses that may be either health-promoting or health-harming responses that may have long-term consequences. Health-harming responses to stress are maladaptive and may cause or exacerbate specific disease states. For example, the

acute stress response of consuming high-energy foods and decreasing physical activity repeatedly is likely to result in overweight or obesity over time. In contrast, positive outcomes such as resiliency and a sense of self-efficacy may develop from successful coping with stressors (Hamill, 2003).

Relevance to present research. Stressors have behavioral, cognitive, and biological effects that can be operationalized and quantified in the laboratory. Animal models are particularly useful because: (1) the stressors and the environment can be controlled; (2) the genetics of the subjects can be controlled; and (3) behavioral and invasive biological measures can be taken regularly.

Figure 1 depicts the conceptual model for the present research project. In this work, the stressor is non-painful restraint. The stress vulnerability construct is manipulated by rat strain (genetically different), sex and body type (individual differences), and housing condition (environment). The dependent variables include behavioral (food consumption, activity), psychological (e.g., simple learning and attention, anxiety-like behavior), and biological (e.g., body weight, stress hormone). The long-term outcomes are not measured in the present project.

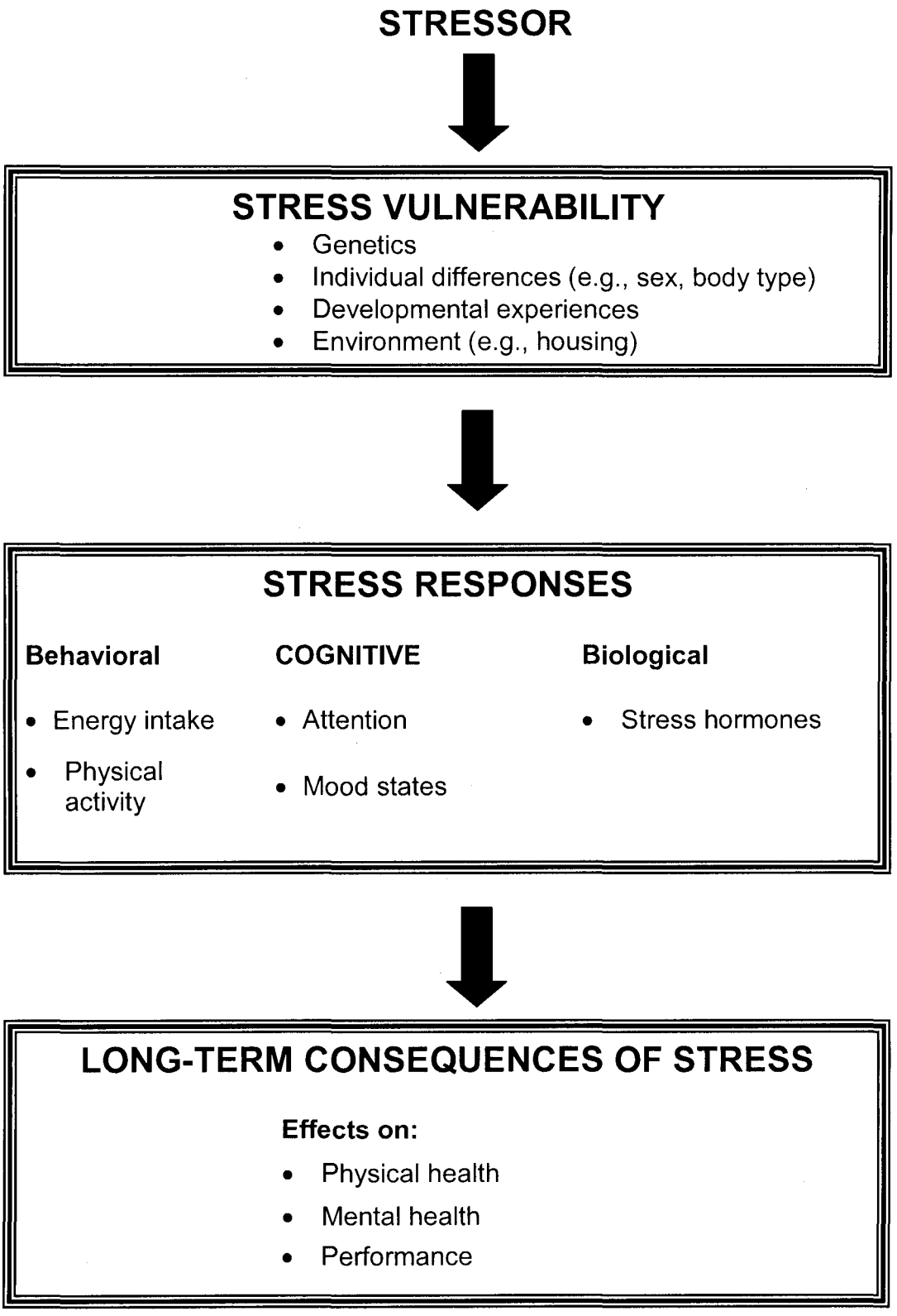

Figure 1. Model of Stress Vulnerability

OBESITY

Definition and Prevalence

Body weight gain results from energy imbalance, which occurs when more calories are consumed than expended (CDC, 2006; Guyton & Hall, 2000). Excess energy is stored in the body as fat. Overweight is an excess of body weight derived from muscle, bone, fat, and/or body water (NIDDK, 2006). Obesity is defined as excess of body fat (Anderson & Wadden, 1999). Overweight and obesity are most often estimated by calculating an individual's body mass index (BMI) or weight (in kilograms) divided by height (in meters) squared (NIDDK, 2006). BMI of 25 - 29.9 kg/m^2 is considered to be overweight and > 30 kg/m^2 is defined as obese (NIDDK, 2006). Obesity has been stratified into three classes (class 1: BMI 30-34.9 kg/m^2; class 2: BMI 35-39.9 kg/m^2; class 3: BMI > 40 kg/m^2) (NHLBI, 1998). The term *obesity* is used throughout this paper to include all three classes. Almost 65% of American adults are either overweight or obese (Flegal, Carroll, Ogden, & Johnson, 2002), 32.2% are obese, and 4.5% are considered extremely obese (Ogden et al., 2006).

Health Relevance

Obesity poses major health risks and is among the leading causes of preventable diseases in the United States (NHBLI, 2000). Obesity is implicated in over 112,000 deaths each year in the U.S. (Flegal et al., 2005). Obesity is often a precursor for medical problems or a cluster of medical complications (i.e., hypertension, insulin resistance, hyperlipidemia) known as metabolic syndrome (Pijl, 2003). The economic burden of obesity as a result of hospitalization, lost worker

productivity, and premature death is estimated at $117 billion annually in the U.S. alone (Stein & Colditz, 2004). Despite a $30 billion weight-reduction industry, the most widely-used treatments for overweight and obesity in the U.S. are largely ineffective (Anderson & Wadden, 1999; Wadden, Brownell, & Foster, 2002).

Rodent models of obesity

Rodent models are widely used to study human obesity because the neuroanatomic (e.g., hypothalamus and limbic) and digestive systems are similar between these species, resulting in shared food preferences and hormonal responses to feeding (Thibault et al., 2004). There are several rodent models of obesity. Genetic components, biological processes, or environmental conditions may be altered to induce excess body weight in animals. One genetic mutation in the fatty allele separates the obese Zucker (fa/fa) from the lean Zucker (Fa/?) (Duclos et al., 2005; Greenwood, Cleary, & Hirsch, 1979). The expression of this single gene mutation is an obese Zucker rat that accumulates 20 - 30 times more fat than does the lean Zucker rat (Greenwood et al., 1979). The fatty strain has a mutated leptin receptor gene (Duclos et al., 2005). This gene produces the hormone leptin which is expressed in adipose tissue (Kandel, Schwartz, & Jessel, 2000; Martinez, 2000; Tataranni, 1998). Leptin provides a neurohormonal signal to the hypothalamus about the quantity of fat in the body. Administering exogenous leptin decreases food intake, increases energy expenditure, and decreases body weight (Clegg, Riedy, Smith, Benoit, & Woods, 2003). Obese individuals have elevated leptin levels, leading researchers to believe that obesity is a result of leptin resistance (Weinsier et al., 1998). The fatty Zucker rat characteristically is

hyperphagic and is commonly used in genetic models of obesity and related metabolic disorders, such as insulin resistance and diabetes (Durham & Truett, 2006; Greenwood & Winocur, 2005; Liu, Mizuta, Kurose, & Matsukura, 2002). Similar to obese humans, obese Zucker rats have high circulating levels of leptin, insulin, glucose, and free fatty acids (Liu et al., 2002; Mattsson et al., 2003). The HPA axis plays a role in the development of obesity in humans and rats. In rodents, excising the adrenal gland reduces body weight and glucocorticoid supplementation increases body weight (Levin et al., 2000). The fatty Zucker rat also has high levels of basal adrenocorticotrophin (ACTH) (Walker, Scribner, Stern, & Dallman, 1992) and corticosterone (Duclos et al., 2005) and produces excess corticosterone in response to stress (Guillaume-Gentil et al., 1990; Mattsson et al., 2003).

Diet-induced obesity is another common animal model of obesity. Animals are fed diets rich in fat or carbohydrates. In some studies, groups are divided by the distribution of body weights after a specified time on a high caloric diet (Levin & Dunn-Meynell, 2000; Tulipano, Vergoni, Soldi, Muller, & Cocchi, 2004). Sprague-Dawley rats fed a moderate high-fat diet (20% fat content) for 10 weeks weighed more and consumed more calories than Sprague-Dawley rats fed a standard pellet chow (3% fat content) (Tulipano et al., 2004). Levin and Dunn-Meynell (2000) reported no difference in food consumption between diet-induced obese rats and control rats, despite a marked 20% difference in body weight. The difference in body weight was attributed to greater metabolic efficiency (or fewer calories to sustain daily functions) and to greater adipose tissue in diet-induced obese rats (Levin & Dunn-Meynell, 2000). Other methods to induce obesity in animals include

surgically altering the ventromedial hypothalamus which causes excessive eating and weight gain (Greenwood & Johnson, 1975; Thibault et al., 2004).

Relevance to present research. The present research focused on stress responses in obese and non-obese rats because excessive body weight is so widespread in the U.S. today and because stress responses may differ in obese and non-obese individuals. Rat models are useful because genetics (Zucker vs. Sprague-Dawley, male vs. female) and environment (food types and housing condition) that influence body weight can be manipulated and controlled.

RATIONALE FOR RESEARCH

Although the existing literature on rat models of obesity is extensive, the findings on whether obesity alters the biobehavioral responses to stress are sparse and contradictory. Methodological differences such as model of obesity (diet-induced, genetically induced, or surgically-induced obesity) and type and intensity of stressor may contribute to the contradictory findings. Previous experiments have been confounded by diet-dependent effects, which prevent causal attributions to body weight. The present research addressed the question of whether stress responses (including behavioral, cognitive, and biological responses) differ in obese and non-obese, male and female subjects living in controlled housing conditions. In order to address this large question, two separate experiments were conducted. Experiment I was conducted to determine the most feasible rat model of obesity. Experiment I included two plausible models of obesity (genetically-based and diet-induced) but only one sex (male) and an A-B-A (A = no stress, B = stress) design. The Baseline, Stress, and Post-stress Phases lasted 10, 17, and 14 days,

respectively. Experiment I revealed that genetically-based obesity creates the clearest model in a short period of time. Experiment II, therefore, used a genetically-based obesity, included male and female subjects, and was conducted as a mixed design experiment that included unstressed control groups.

OVERVIEW OF WORK

Two separate experiments were included in this doctoral dissertation research to examine the biobehavioral effects of repeated stress as a function of body weight, diet, and sex. Experiment I examined behavioral and biological effects of repeated acute stress on obese (Zucker) and non-obese (Sprague-Dawley) male rats. Experiment I was conducted to: (1) allow the investigator to gain experience with the many independent and dependent measures involved in this work; (2) determine the best rat model of obesity to use (i.e., genetic-based or diet-induced) in Experiment II (which included male and female rats in stressed and unstressed conditions). The best rat model of obesity was characterized by marked weight gain (> 100%).

Experiment II examined behavioral and biological effects of repeated acute stress in *male and female* genetically obese and non-obese rats. All animal procedures were approved by the Uniformed Services University Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub., 82-23, rev.1985).

SECTION II: RESEARCH EXPERIMENTS

EXPERIMENT I

Experiment I examined differences in biobehavioral responses of genetically and diet-induced obese male rats to repeated exposure to a mild physical stressor. The experiment was conducted in three experimental phases using an A-B-A design. Each experimental phase corresponded to a change in the stress condition (A = no stress, B = stress). All animals were exposed to behavioral testing before, during, and after a 17-day period of repeated acute restraint stress. The entire experiment lasted for 58 days. Differences were examined by Strain, Food, Time, and interaction of these variables (i.e., Strain X Food, Strain X Time, Food X Time, and Strain X Food X Time). See Appendix 1, Table 1, for timeline.

Many of the dependent variables (e.g., food consumption, body weight, activity, and acoustic startle response) were measured repeatedly over the course of the experiment. Repeated measurements allowed determination of the effects of stress over time. Some behavioral dependent variables required acclimating animals to the testing procedures. Acclimation minimized effects that may be associated with stress related to novelty or routine handling.

Rationale for Independent Variables Relevant to Experiment I

Experiment I used two strains of male rats (Sprague-Dawley and Zucker) exposed to mild, repeated immobilization stress. The behavioral dependent measures were divided into three domains: (1) behaviors (feeding and activity); (2) cognitive processes (attention, anxiety, and pain); and (3) biological measures (body weight and the primary stress and energy-regulating hormone, corticosterone). The

goal of using this particular combination of dependent measures was to characterize any differential stress response patterns that may exist between obese and non-obese rats.

Rat Strain

Sprague-Dawley. The Sprague-Dawley rat is commonly used in animal models to examine various biobehavioral responses (Costa, Smeraldi, Tassorelli, Greco, & Nappi, 2005; Grunberg, Popp, & Winders, 1988; Harris et al., 1998; Khasar, Green, & Levine, 2005; Levine & Morley, 1982; Michel et al., 2003). Phenotypically, Sprague-Dawley rats are albinos with white coats and unpigmented retinas (Charles River Laboratories). This strain is not altered genetically or bred for any unique characteristics.

Fatty Zucker. The obese Zucker rat was a serendipitous finding in 1961 (Zucker & Zucker, 1961). In three separate litters from the same parents, the occurrence of at least one obese offspring was about 25% (Zucker & Zucker, 1961). The fatty Zuckers are pigmented typically with a black or brown and white coat. The obese Zucker rat becomes noticeably larger than normal weight control rats at approximately 21 - 22 days old (Durham & Truett, 2006; Zucker & Zucker, 1961). At this time, the obese Zucker rat begins to consume large amounts of food and is characterized by hyperinsulinemia. Based on these unique characteristics, obese Zucker rats have been extensively used in experimental studies that examine obesity and insulin resistance. The terms *fatty* and *obese are* used interchangeably in this write-up to refer to the obese Zucker (fa/fa) strain.

Diet

Standard chow. Standard chow provides a well-balanced macronutrient content and consists of 25% protein, 4% fat, and 5% fiber (Harlan Teklad). The standard chow diet provides about 3 kilocalories per serving. The term *bland chow* also is used interchangeably in reference to standard chow.

Cafeteria diet. In addition to genetic manipulation, diet can be manipulated to increase body weight. A "cafeteria diet" usually increases eating and body weight in rats. A cafeteria diet (that includes a variety of foods that are eaten by humans) also more accurately reflects the eating behavior and food preferences of humans than meals such as sucrose-flavored solutions (Bell et al., 2002) or vegetable shortening (Harris et al., 1998). Rats fed a high-fat diet, which consists of 20% vegetable shortening and 80% standard chow, gain more weight than do rats fed standard chow alone (Harris et al., 1998). Similar findings have been reported on a cafeteria-style diet that consisted of 33% standard rat chow, 33% sweetened condensed milk (Nestle™), 7% sucrose, and 27% water (Holemans, Caluwaerts, Poston, & Van Assche, 2004).

Sclafani and Springer (1976) were the first to use a cafeteria diet such as marshmallows, cookies, milk chocolate, and salami in animal models of obesity. A cafeteria-style diet consists of highly palatable foods that are high in fat and carbohydrates. These highly palatable food choices are consistent with the assortment of foods available to humans. When foods high in fat and sugar are provided in addition to the standard, nutritionally balanced chow diet, intake of palatable foods increases (Dallman et al., 2003). Overeating also occurs by 20 -

40% compared to a diet of standard chow alone (Sclafani, 2001). Diet-induced obese animals also are less likely than chow-fed animals to work for food (Sclafani & Springer, 1976). The increased consumption of highly palatable foods and decreased amount of physical activity levels among rats fed cafeteria diet lead to an increase in body weight. Marked differences in body weight between rats fed a cafeteria diet and rats fed bland chow are present after two weeks (Lauterio, Davies, DeAngelo, Peyser, & Lee, 1999). Animals in Experiment I were fed Oreo™ cookies and Lay's™ potato chips based on previous findings in our laboratory that rats offered these foods consumed substantial amounts (Grunberg et al., 1988; Tomchesson, 2006). In addition to these high-energy foods, standard chow was available to ensure that rats had access to a nutritionally-balanced meal. Because diet-induced obese rats have similar metabolic disturbances as do humans, such as insulin resistance (Levin et al., 2000), the findings from this research project are relevant to humans.

In humans and rats, it has been suggested that high-fat foods have an increased caloric density which, in turn, promotes palatability of the foodstuff and overeating (Rolls, Drewnowski, & Ledikwe, 2005; Warwick, Synowski, & Bell, 2002). Experiment I provided rats with a high-energy diet which is relatively high in fat, sugar, and simple carbohydrates.

A growing body of research suggests that macronutrients affect behavioral responses. Prasad and colleagues (1996) pre-tested male Sprague-Dawley rats for anxiety responses to the elevated plus maze and retested them after being fed 90% of their total kilocalories either from protein, carbohydrates, or fat for 7 days. Rats

fed high-protein and high-carbohydrate diets had no difference in anxiety responses to the elevated plus maze between the pre-test and retest, but rats fed a high-fat diet had significantly reduced anxiety responses (Prasad & Prasad, 1996). Rats fed diets high in protein and carbohydrates did not have significant reductions in anxiety responses (Prasad & Prasad, 1996). Rats exposed to cold stress consumed greater amounts of a sucrose solution than when at room temperature (Bell et al., 2002). Male Wistar rats exposed to 1-hour restraint stress, 5 days a week for 50 days consumed more sweet-tasting food (Froot Loops cereal) in fasted and nonfasted states (Ely et al., 1997). These findings imply that specific macronutrients may attenuate responses to stressful situations.

RATIONALE FOR STRESS MANIPULATION

Immobilization or restraint stress. There are several animal models to induce the sequela of stress-related responses. Chronic mild stress paradigms introduce various mild stressors, such as cage shaking and tilting, in a repeated and unpredictable manner (Kim & Han, 2006). Other stress paradigms include immobilization or restraint (Faraday, Blakeman, & Grunberg, 2005; Faraday, O'Donoghue, & Grunberg, 1999; Raygada, Shaham, Nespor, Kant, & Grunberg, 1992), crowding (Brown & Grunberg, 1995), social defeat (Moles et al., 2006), immersion in cold water (Bell et al., 2002), and mild electric shock (Estanislau & Morato, 2006). Both the terms *immobilization* and *restraint* have been used in reference to the type of stress manipulation procedure used in this research project.

Immobilization is a widely used manipulation to evoke biobehavioral responses to stress in animal models (Faraday et al., 2005; Faraday et al., 1999; Gamaro et al., 1998; Raygada et al., 1992; Tannenbaum et al., 1997). This type of stress manipulation is considered to be a mild stressor because of the relatively short recovery of stress-induced physiological changes in comparison to more severe stressors (e.g., high intensity inescapable footshocks) (Garcia et al., 2000). Rats produce substantial elevations in stress hormones such as ACTH and corticosterone after exposure to restraint stress (Tannenbaum et al., 1997; Gameiro et al., 2006; Faraday et al., 2005). In response to a single, 20-minute exposure to restraint stress, there is a three-fold rise in ACTH and corticosterone levels (Schrijver, Bahr, Weiss, & Wurbel, 2002) that remits within 1-3 hours post-stress exposure (Garcia, Marti, Valles, Dal-Zotto, & Armario, 2000; Schrijver et al., 2002). This repeated, mild stressor provides a model of daily or frequent stressors experienced by humans rather than a single traumatic event.

Relationship among restraint stress and body weight, food consumption, and activity level. Significant differences in body weight, food consumption, and activity levels can be detected with daily exposures to 20 minutes of immobilization for 14-21 consecutive days (Faraday, 2002; Faraday et al., 2005). Male rats exposed to immobilization stress for 3 hours on three consecutive days lost a small but statistically significant 5 to 15% of body weight (Harris et al., 1998). These significant reductions in body weight persisted for 40 days after the stress period in male rats repeatedly restrained for 3 hours over the course of 3 days (Harris et al., 1998). In another study, 21 days of restraint stress for 6 hours per day decreased

exploratory behaviors in an open-field in male Sprague-Dawley rats (Conrad, LeDoux, Magarinos, & McEwen, 1999).

RATIONALE FOR DEPENDENT VARIABLES RELEVANT TO EXPERIMENT I

BIOLOGICAL MEASURES

Body weight (BW)

Body weight (BW) is a simple measure that has been widely used as an index of health status (e.g., Brown & Grunberg, 1995; Grunberg, 1982). The rodent literature usually describes subjects according to body weight in grams. Healthy rats typically gain weight from birth to about 15 weeks (Charles River Laboratories; Wilmington, MA). Taking body weight of rats is a well-established measure in our laboratory (Faraday, Blakeman, & Grunberg, 2005; Grunberg, 1982; Grunberg, Bowen, & Winders, 1986; Saah, Raygada, & Grunberg, 1994; Winders & Grunberg, 1990).

Lee index (LI)

The direct method to measure body fat among rats is by carcass analysis (Simson & Gold, 1982). Similar to BMI in humans, the Lee Index (1929) is an alternate, rudimentary method to estimate body fat and has been used in recent work conducted in our laboratory (Tomchesson, 2006). The correlation between carcass analysis and the Lee Index is high for diet-induced obesity in rats (Simson & Gold, 1982). Measurements obtained from the Lee Index are most reliable when performed on sedated animals (Simson & Gold, 1982). The "height" of the rat is measured from the tip of the nose to the beginning of the tail (nasal-anal length).

This research project used the formula presented by Lee (1929) to calculate the LI scores: LI = [(g body wt) $^{1/3}$ / (mm body length)] X 10^4.

Corticosterone

Corticosterone, a glucocorticoid, is essential to ensure relatively stable levels of circulating glucose during normal cycles of feeding, resting, and activity – events that vary in terms of energy demand and expenditure. Corticosterone is also the primary stress hormone and is critical to maintain energy availability and allow the individual to resist and cope with physical, metabolic, and psychological stressors (Guyton & Hall, 2000). Corticosterone levels increase in response to stress. Trunk blood was taken at the end of the experiment to assess corticosterone levels.

BEHAVIORAL MEASURES

Food consumption (FC)

In humans and rats, food consumption is another behavioral marker of health status. For example, change in appetite is a criterion for clinical depression (APA, 2001). The emotional eating literature suggests that a subgroup of individuals consume food in response to negative affect, whereas there are others who eat less in response to negative affect (Gibson, 2006). Male rats exposed to the tail pinch (Greeno & Wing, 1994; Morley, Levine, & Rowland, 1983; Levine & Morley, 1981) and repeated cold stress (Schultz, Collier, & Johnson, 1999) increased food intake. Male and female rats exposed to restraint stress (Zylan & Brown, 1996; Faraday, 2002) decreased feeding. Food consumption is a well-established measure in our laboratory (Brown & Grunberg, 1996; Grunberg, 1982; Grunberg et al., 1986;

Winders & Grunberg, 1990). Consumption was determined by subtracting remaining food weight from previous food weight.

Physical activity

Physical activity is frequently used to asses the effects of stressors in rodents.

Home cage activity (HCA). Home cage activity is defined as behavior that occurs in the housing environment. This measure is unlike the other behavioral measures that were used in this experiment because it involves naturalistic observation of the animal in its primary living space. Home cage activity was measured to determine if stress altered behaviors in the living environment. The observation technique used in this research was based on a technique that was recently developed and modified in our laboratory (Tomchesson, 2006).

Open field (OF). Open-field locomotion describes an animal's behavior when it is removed from its home cage environment and placed in an unfamiliar arena. This measure yields information about the amount of horizontal and vertical activity. Open-field locomotor activity is a well-established measure of physical activity (e.g., horizontal and vertical) and general health in our laboratory (Bowen, Eury, & Grunberg, 1986; Grunberg & Bowen, 1985). Previous investigators have found that locomotor activity remains similar across repeated testing sessions among control subjects and that stress changes locomotor activity (e.g., Acosta & Rubio, 1994; Conrad, LeDoux, Magarinos, & McEwen, 1999; Faraday, 2002; McCormik & Ibrahim, 2007). Changes in locomotor activity were used in the present experiment to interpret the effect of stress on physical activity. Exploration (e.g., vertical activity) and general movement (e.g., horizontal activity) are different types of locomotion

variables that can be affected by stress. Greater levels of horizontal and vertical activity are consistent with adaptive stress responses.

COGNITIVE MEASURES

In humans and rodents, responses to stress may be adaptive or maladaptive. The present research exposed rats to restraint stress in order to examine stress reactivity in obese and non-obese rats. The behavioral measures provided information about cognitive processes and are described below. Indices of anxiety (e.g., center time in the open field, elevated plus maze) and nociception (e.g., hot plate) were measured in the proposed work; these data did not yield findings, add little to the overall project, and are not presented.

Acoustic startle response (ASR) with and without pre-pulse inhibition (PPI)

Acoustic startle response (ASR) and pre-pulse inhibition (PPI) have been used in our laboratory to index the effect of stress on cognitive processes (Acri, 1992, 1994; Acri, Grunberg, & Morse, 1991; Faraday et al., 1999). In humans and rats, ASR is a behavioral indicator of unconditioned responding to auditory stimuli (Davis, 1984). Startling in response to a loud noise is reflexive, but the startle response can be attenuated when the loud noise is preceded by a nonstartling cue or pre-pulse (Faraday et al., 1999). This reduced response is known as pre-pulse inhibition. PPI is interpreted as an attentional response or index of information gating. The startle response is largely controlled by the brainstem. However, because other neural structures associated with higher cognitive functioning (e.g., hippocampus, amygdala, cingulate gyrus) contribute to this response, the startle amplitude is a marker of attention and emotional states (Acri, 1992, 1994; Anthony & Graham, 1983; Bradley, Cuthbert, & Lang, 1990; Simons & Zelson, 1985; Swerdlow,

Caine, Braff, & Geyer, 1992). If the animal is able to discriminate between relevant and irrelevant environmental stimuli, then PPI is intact (Swerdlow et al., 1992). An adaptive response to a pre-pulse is a decrease in startle amplitude. When an increase in startle or no change in startle is accompanied by a decrease in the amount of or percent of pre-pulse inhibition, the interpretation is an impairment in attentional processing. ASR with and without pre-pulse were included in this research project to assess stress-induced changes to acoustic stimuli and sensory-gating or attention (PPI).

Experimental Design and Sample Size

Experiment I was a 2 X 2 X 3 full factorial mixed design with between-subjects factors of Strain (Sprague-Dawley or Zucker) and Diet (bland chow only or bland chow plus cafeteria diet) and within-subject factor of Time (before, during, and after stress). There were 10 subjects per cell for a total of 40 subjects.

The number of subjects was calculated based on sample sizes used in previous experimental studies with Sprague-Dawleys using similar dependent variables and estimated power of 0.80 using alpha level of 0.05 (two-tailed tests). Effect size was determined by calculating an estimated omega squared and using phi statistics. Using phi and power tables, the minimal sample size required to achieve power of 0.80 was calculated. A minimum of 6 subjects per cell was necessary to detect differences in stress responses based on previous experiments in our laboratory, review of the literature, and these calculations. Because studies using Zuckers and similar dependent variables have not been conducted, the number of subjects per cell was increased to 10 subjects per cell.

SPECIFIC AIMS, HYPOTHESES, AND DATA ANALYTIC STRATEGY: EXPERIMENT I

The specific aims of Experiment I were: (1) to determine the most feasible animal model of obesity, and (2) to compare stress responses in obese and non-obese rats. Experiment I examined the effects of repeated acute stress responses on body weight and composition, feeding, energy regulation, and cognitive processes in obese and non-obese male rats from two different strains. The central hypothesis of this experiment was that obese rats would have more maladaptive behavioral and biological responses to stress than non-obese rats. The specific aims and hypotheses are described below. The following abbreviations are used in reference to the four experimental groups: Sprague-Dawley/Bland diet (SB), Zucker/Bland diet (ZB), Sprague-Dawley/Cafeteria diet (SC), or Zucker/Cafeteria diet (ZC). The term *obese* refers to genetically and diet-induced obese rats.

The goals of data analyses were to determine the extent to which feeding, body weight, and biobehavioral responses to stress differed between genetically obese and diet-induced obese rats. All tests were two-tailed with $p < 0.05$. Several strategies were used to reduce the probability of Type I error (Keppel, 1991). First, the experiment was designed with power of 0.80. Second, internal analyses were conducted only if the overall analyses revealed significant main effects or interactions. Third, multivariate analyses of variance were used for intercorrelated variables. Fourth, the within-subject error term relevant to the comparison group was used instead of the error term for all subjects.

Specific Aim #1: Determine the most feasible model of rodent obesity

Hypothesis 1a. The genetically-induced model of obesity will produce rats heavier in body weight more quickly than will the diet-induced model of obesity.

Rationale. Sprague-Dawleys are typically used as lean control rats because they are not altered genetically or bred for unique characteristics. The obese Zucker rat becomes noticeably larger than normal weight control rats at approximately 21 - 22 days old (Durham & Truett, 2006; Zucker & Zucker, 1961).

Hypothesis 1b. Genetically obese rats fed cafeteria food will consume more food than all other groups (ZC > ZB $\geq$ SC > SB).

Rationale. Overeating occurs by 20% - 40% when palatable foods are offered compared to when standard chow alone is offered (Sclafani, 2001). The interaction between genetics and consuming calorically dense, palatable foods was expected to create obese rats at a faster rate than genetics or a high-caloric diet alone.

Specific Aim #2: Determine biobehavioral responses to stress in obese and non-obese rats

Hypothesis 2a. Genetically obese rats will gain more weight and consume more food than other groups in response to stress (ZC > ZB $\geq$ SC > SB).

Rationale. Obese stressed rats fed a medium-fat diet gained more weight than non-obese stressed rats fed the same diet without an associated increased in food consumption (Michel et al., 2003). However, this particular study did not use palatable foodstuffs. When palatable foods, such as Froot Loops® cereal (Ely et al., 1997) or other sweet-tasting foods such as a sucrose solution (Bell et al., 2002)

were provided to stressed rats in previous studies, consumption of these foods increased. It was predicted that consumption of the palatable foods in the present experiment (e.g., chips and cookies) would increase in response to stress.

Hypothesis 2b. Stress will reduce physical activity to a greater extent among obese rats than lean rats (e.g., decreased horizontal activity, vertical activity and decreased activity in the home cage) and will increase startle and decrease percent PPI more among obese rats than lean rats.

Rationale. Levin and colleagues (2000) reported no significant differences between obese and non-obese rats in open field activity after exposure to stress, but obese rats were generally less active. Michel and colleagues (2003) reported that obese stressed rats had greater horizontal activity than did non-obese stressed rats during the first 7 minutes in the open field. The weakness of these previous studies is that activity in the open field was only measured for 10 and 15 minutes, respectively. Overall physical activity cannot be determined from these short observation periods because the animal is adapting to the novel testing environment. These studies did not control for novelty effects. The present experiment controlled for novelty effects with an acclimation phase that preceded baseline measurements. Animals were acclimated to handling (e.g., gentling animals for 3 days) and to the testing equipment (e.g., placing animals in open field chamber for two 1-hour sessions). Therefore, it was expected that rats fed a rich-fat diet would result in more adaptive stress responses as measured by increased physical activity.

To this author's knowledge, no studies have examined differential stress responses in obese and non-obese rats on acoustic startle response and percent pre-pulse inhibition. The central hypothesis of the present experiment was that obese rats will have more maladaptive responses to stress. A greater response to the pre-pulse than to the startle is maladaptive.

Hypothesis 2c. Obese rats will have a greater acute stress response as indicated by higher corticosterone levels than others (ZB > ZC ≥ SB > SC).

Rationale. Obese Zucker rats produced higher amounts of corticosterone in response to a single 1-hour period of immobilization than lean Zucker rats (Guillaume-Gentil et al., 1990). Obese Zucker rats also produced higher amounts of corticosterone with subsequent repeated stressors compared to lean rats (Guillaume-Gentil et al., 1990). To this author's knowledge, no studies have compared differences in biological responses to stress between obese Zuckers and lean Sprague-Dawleys. Because other investigators found that a rich-fat diet decreased anxiety-like behaviors in rats, it was expected that the biological response to stress also will decrease (Prasad & Prasad, 1996).

METHODS: EXPERIMENT I

Subjects

Subjects were 20 male Sprague-Dawley rats and 20 male fatty Zucker rats (Charles River Laboratories, Wilmington, MA). Upon arrival, the rats were approximately 20 – 30 days old and weighed 40 - 50 grams. Adolescence in the rat is typically defined as 21 - 55 days (Faraday, Elliott, & Grunberg, 2001; Spear & Brake, 1983). Therefore, subjects were adolescents during the acclimation phase and young adults during the Baseline, Stress, and Post-stress Phases.

Housing

Twenty male Sprague-Dawley rats and 20 male fatty Zucker rats were single-housed in polycarbonate cages (40 cm X 20 cm X 20 cm) in a climate-controlled room maintained at approximately 23°C and 50% relative humidity. All animals were housed on hardwood chip bedding (Pine-Dri) with continuous access to water and standard rat chow (Harlan Teklad 4% Mouse/Rat Diet 7001). The housing room was on a 12-hour reversed light-dark cycle (lights off at 0400 hours; lights on at 1600 hours) such that behavioral testing was conducted during the animals' normal active phase. Upon arrival, animals were acclimated to the housing facility and handled daily for three days for approximately 2 - 3 minutes per day.

PROCEDURES

Table 1 in Appendix A provides a timeline for the procedures used in Experiment I. Pictures of equipment used in the present research project also are included in Appendix A.

All animals had nine days to acclimate to the housing facility, standard chow, and routine handling for behavioral testing prior to being divided into the diet groups. Then, the rats were divided into four weight- and activity-matched groups in a full factorial design: genetic (Sprague-Dawley or Zucker) and diet (cafeteria or bland) groups. In the cafeteria diet condition, animals had access to highly palatable and calorically dense foods (Oreo™ cookies and Lay's™ plain potato chips) in addition to standard chow. The rats either had access to the cafeteria diet or bland chow only diet for 18 days before the stress protocol began (i.e., 8 days were during the acclimation period and 10 days during the Baseline Phase). Marked weight differences between groups were expected to occur within 14 days of providing the palatable foods (Lauterio et al., 1999). All subjects were acclimated to the open-field (OF) activity chambers and acoustic startle response (ASR)/pre-pulse inhibition (PPI) chambers to reduce any stress related to a novel situation (Faraday & Grunberg, 2000). Rats were exposed to two OF testing sessions and three ASR/PPI testing sessions during the acclimation phase. All behavioral measures were conducted between 0700 and 1200 hours (the dark/active cycle). Animals' behavior was evaluated during the active phase (dark/light cycle) to provide data analogous to daytime for humans.

During the Stress Phase, the rats were restrained for 20 minutes, starting at 0700 hours, for 17 consecutive days. Behavioral testing began within 5 minutes after removal from the restrainer. Body weight and food consumption were measured every 2-3 days. The dependent variables were measured for 14 days after the Stress Phase to determine whether responses after stress exposure differed in obese and non-obese rats. Half of the animals from each of the four conditions (n = 20) were randomly selected to be re-exposed to restraint stress. These animals were sacrificed within 5 minutes after re-exposure to restraint. All animals were euthanized with carbon dioxide and decapitated following a sequence that was counterbalanced across the four conditions to minimize effects caused by order or time of day. Trunk blood was collected from all 40 animals to examine corticosterone levels in response to acute stress.

Stress Manipulation

Immobilization. See Figure 37 in Appendix A. Each animal was placed in a finger-like restraining device (Centrap Cage, Fisher Scientific) for 20 minutes on 17 consecutive days based on Raygada et al. (1992) and Faraday (2002). When tightened, the "fingers" enclosed the animal and restricted movement without pinching or causing apparent pain. Within 5 minutes after removal from the restraining device, behavioral testing occurred. The stress manipulation occurred in a dedicated room that was separate from the housing room and from the rooms where behavior testing occurred.

Dependent Variables

Behavioral

Food consumption (FC)

Food consumption was measured two to three times per week providing a total of 21 measurements. All rats had access to standard bland chow, which is specially formulated to provide a nutritionally balanced meal (25% protein, 4% fat, and 5% carbohydrates; Harlan Teklad™). New cookies and potato chips were added two to three times per week to ensure freshness for rats fed a cafeteria diet. The Lay's™ potato chips (28 grams per serving) had 150 kilocalories and contained 10 grams of fat, 2 grams of protein, and 15 grams of carbohydrates. The Oreo™ cookies (34 grams per serving) had 160 kilocalories and contained 7 grams of fat, 2 grams of protein, and 25 grams of carbohydrates.

Physical activity

Two different methods of behavioral observation were used to assess physical activity. The first method was home cage activity (HCA). The second method was open field (OF; locomotor).

Home cage activity (HCA). See Figure 38 in Appendix A. Activity level and type of activity in the home cage were observed for two 30-second intervals. At the end of each 30-second interval, the level of activity for each subject was rated on a 7-point scale (1-none; 7-constant high activity). The number of animals engaged in the following activities also were recorded by condition: feeding, grooming, awake/not moving, horizontal or vertical movement, and sleeping. HCA was measured during acclimation, Baseline, Stress, and Post-stress Phases for a total of

four measurements. Acclimation data were not included in data analyses. Two raters provided independent ratings at different time points on the same observation day. The rationale for conducting the ratings at different time points was to observe animal behavior over a duration of time. All raters were experienced raters from previous studies that used a similar version of the HCA measure. The experimenter provided additional training on the revised HCA measure.

Open-field or Locomotion. See Figure 39 in Appendix A. Locomotor activity was measured using an Omnitech Electronics Digiscan infrared photocell system (test box model RXYZCM [16 TAO]; Omnitech Electronics, Columbus, OH) in a dark room designed to reduce outside sound. Animals were placed in a 40 cm X 40 cm X 30-cm Plexiglas chamber with a lid that has multiple 3.5-cm diameter ventilation holes. A photocell array measured horizontal activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the arena floor. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measured vertical activity. Data were transmitted to a computer analyzer. A computer interface automatically collected animal data every 5 minutes for a total testing period of 1 hour. Acclimation to the open-field apparatus consisted of two separate 1-hour testing sessions (Bowen et al., 1986; Grunberg & Bowen, 1985); Faraday, 2002).

Horizontal activity and vertical activity were used to measure physical activity in an unfamiliar environment. It is important to determine whether any stress-induced changes in body weight were associated with activity levels because most

of the experimental variables were dependent on physical movement. A total of ten OF measurements were taken (two during acclimation, one during Baseline Phase, four during Stress Phase, and three during Post-stress Phase). Each OF testing session lasted 1 hour. Alcohol (35% ethanol) was used to clean the OF chamber between each testing session.

Biological

Corticosterone (CORT). Serum corticosterone level was measured as a biological marker of the stress response. Half of the animals from each condition were re-exposed to restraint stress approximately 25 minutes prior to decapitation. Trunk blood was collected to determine differences in acute stress responses between obese and non-obese rats. The blood was spun in a centrifuge for 20 minutes to separate the plasma from the serum and serum was stored at -80°C for later assay.

At the end of this experiment, animals were euthanized by inhalation of carbon dioxide. Then, each animal was decapitated using a standard rodent guillotine (4.5 inch blade), blood samples were collected from the trunk, and tissue samples were removed and stored for other investigations. Blood samples were transferred to microcollection tubes, placed on ice for 20 minutes, centrifuged at 3000 RPM for 15 minutes to separate plasma from serum, and serum was transferred to other tubes and stored at - 80°C for later assay.

Serum Corticosterone Extraction Process. Serum corticosterone was assayed by an ImmuChem Double-Antibody radioimmunoassay (RIA) kit using ^{125} I-labeled corticosterone (MP Biomedicals, Orange Park, NY). A limited amount of

specific antibody is reacted with a fixed quantity of ^{125} I-labeled corticosterone. The concentration of unlabeled corticosterone in samples increases as a function of the decreasing percentages of bound radioisotope-labeled corticosterone. A second antibody precipitates antibody bound to antigen. The quantity of endogenous corticosterone was determined by measuring the radioactivity of the precipitate with known standards from the same assay in a gamma counter and converting disintegration per minute (DPM) into concentrations. All samples and standards were run in duplicate. The sensitivity of the assay is 8 ng/ml (MP Biomedicals, Orangeburg, NY). The intra-assay and inter-assay coefficients of variation are 10.3% and 7.1%, respectively. This measure is included to verify that the stress manipulation activated the HPA axis and to assess any differences in biochemical stress responses.

Body weight (BW)

Each animal was removed from its cage and placed on an electronic scale to obtain body weight (BW). To reduce movement artifacts, the electronic scale automatically takes ten weight readings in rapid succession and then provides an average of these readings. A total of 22 BW measurements were taken over the course of Experiment 1 (four during acclimation, three during baseline, nine during stress phase, and six during Post-stress phase).

Psychological Measures

Acoustic startle response (ASR) with and without pre-pulse inhibition (PPI). See Figure 40 in Appendix A. ASR and PPI were conducted using a soundproof chamber with a platform to detect changes in weight (MED-ASR-310; Med

Associates, Georgia, VT). The platform was centrally located from the speakers inside the floor and ceiling of the chamber. Each animal was placed in an 8 cm X 8 cm X 16 cm open air cage that fits atop the weight-sensitive platform. The open air cage permitted the animal to make small movements such as turning around. Following placement of animals in the chambers, there was a 3-minute adaptation period during which no startle stimuli were presented. Distractions in the testing room were masked by providing 56 dB of ambient background noise. Movements in response to acoustic stimuli are measured as voltage change by a strain gauge inside each platform. Responses were computer-recorded as the peak response occurring during the no-stimulus, pre-pulse, and startle periods.

Startle stimuli consisted of 110 dB or 120 dB noise bursts that lasted for 20 msec. A softer noise or pre-pulse of 68 dB or 82 dB the louder noise bursts (i.e., 110 dB or 120 dB) preceded by 100 msec. These particular decibels were selected as acoustic stimuli based on previous work that consistently produced a startle response (Acri, 1994; Acri, Grunberg, & Morse, 1991; Faraday, 2002). Each startle stimulus began and ended suddenly. Six different types of stimulus trials were presented eight times (48 trials total) in an unpredictable manner to reduce order effects and habituation. The trial types included: (1) 110 dB stimulus, (2) 110 dB stimulus preceded by a 68 dB pre-pulse, (3) 110 dB preceded by a 82 dB pre-pulse, (4) 120 dB stimulus, (5) 120 db stimulus preceded by an 68 db pre-pulse, and (6) 120 dB stimulus preceded by an 82 dB pre-pulse (Faraday, 2002). Inter-trial intervals ranged randomly from 10-20 sec.

The testing period lasted approximately 15 minutes. Holding cages were washed with warm water and dried after each use. Alcohol (35% ethanol) also was used to clean the holding cages in order to mask any olfactory cues that may signal distress to animals subsequently tested. Each sound-attenuated chamber was allowed to ventilate with the door open for about 10-15 minutes between animals to optimize clearance of any alarm pheromones that might be present in the chamber. Two animals per condition were tested in each run in a counterbalanced manner.

Based on previous findings, animals produce reliable ASR and PPI responses after one to three separate exposures to the startle protocol (Faraday & Grunberg, 2000). A total of eight ASR measurements were taken (three during acclimation, one during baseline, three during Stress Phase, and one during Post-stress Phase).

Each animal's responses were averaged within trial type. Percent pre-pulse (%PPI) was calculated as [(amplitude of trial without pre-pulse) – (amplitude of trial pre-pulse)/amplitude of trial without pre-pulse] X 100. The product was analyzed as %PPI. These calculations were based on established procedures of several investigators (Acri, 1994; Acri, Grunberg, & Morse, 1991).

RESULTS: EXPERIMENT I

General Data Analytic Approach

The following abbreviations are used in reference to the four experimental groups: Sprague-Dawley/Bland (SB), Zucker/Bland (ZB), Sprague-Dawley/Cafeteria (SC), or Zucker/Cafeteria (ZC). The term *cafeteria diet* is used throughout this paper in reference to rats fed standard chow, cookies, and chips. The term *bland chow only* is used in reference to rats that were not fed cookies or chips in addition to standard food.

Because several measurements were taken for each dependent variable, the following strategy was used to collapse the data for interpretation by phase. The last measurement taken prior to the start of restraint stress was used as baseline. Then, the group mean was calculated and graphed for every measurement taken (see appendix for descriptives and graphs). The graphs were examined for patterns of activity. Because there were consistent patterns of activity, specific measurements for each dependent variable were selected to represent the Stress Phase and Post-stress Phase. The criteria used to select the specific time points were as follows: (1) sufficient time elapse to capture the effects of stress and recovery from stress, and (2) relatively close temporal proximity of data collection for most dependent variables during each phase to control for effects of time and maturation and to allow comparison of activity across measures.

Significant global MANOVA results determined whether to examine subgroups separately. Sub-group analyses followed only if overall analyses revealed significant main effects or interactions. Separate univariate analyses of

variance (ANOVAs) for each variable were performed. Only ANOVAs are reported. Univariate ANOVAs were conducted to determine if the groups differed at baseline and on specific measurement days. If there were differences, then baseline measurements were used as a covariate. Repeated-measures analyses of variance (ANOVAs) were conducted to determine the effect of stress on groups over time, with Strain and Diet as the between-subjects factors and Time as the within-subject factor. Paired samples t-tests were used to detect differences within groups between phases (see Appendix A). Independent samples t-test were used to detect differences between strains and diet groups. Tukey HSD *post hoc* test of multiple comparisons was used when there was a significant Strain X Diet interaction in order to detect differences among more than two groups. All graphed data are group means and standard error of the mean. Only significant findings are reported.

Body weight. Because body weight differences were the primary variable of interest, body weight analyses were performed both with and without baseline values as covariates. This approach allowed the examination of data with and without controlling for initial body weight differences. The last body weight measurement taken prior to the start of restraint stress was used as baseline (Baseline Day 10). Body weight measurements taken on Stress Days 13 and 15 were averaged into a two-day period to represent the mean body weight during the Stress Phase and analyses were conducted on the mean. Body weight measurements taken on Post-stress Days 7 and 9 were averaged into a two-day period to represent the mean body weight during the Post-stress Phase and analyses were conducted on the mean.

Corticosterone. ANOVAs were used to analyze the corticosterone data.

Lee Index. ANOVAs were used to analyze the Lee Index data.

Food consumption. Because body weight is related to food consumption, and body weight was the primary variable of interest, food consumption analyses also were performed both with and without baseline values as covariates. This approach allowed the examination of data with and without controlling for initial feeding differences. The last food measurement taken prior to the start of restraint stress was used as baseline (Baseline Day 10). Because food consumption was measured every 2-5 days, adjustments were made to calculate the average amount of daily food consumption. Food consumption measurements taken on Stress Days 13 and 15 were averaged to represent the mean amount of food consumed during the Stress Phase and analyses were conducted on the mean. The amount of food consumed on Post-stress Days 7 and 9 were averaged to represent the amount of food consumed during the Post-stress Phase.

Separate analyses were used to analyze the food consumption data converted to grams and to kilocalories consumed. The total amount of kilocalories consumed at baseline, during the Stress Phase, and during the Post-stress Phase was calculated using the following formula:

Calories from chow + calories from chips + calories from cookies

Home Cage Activity. Four separate levels of home cage activity ratings (conducted by two different raters) were measured each phase (baseline, stress, and post-stress) for each animal. These four ratings were averaged together and the mean represented the level of home cage activity for the respective phase.

Separate univariate ANOVAs were used to detect differences between groups during the Baseline, Stress, and Post-stress Phases on level of home cage activity.

Non-parametric statistical analyses were used to measure engagement in specific types of activities. Four separate observations (conducted by two different raters) tallied the number of animals engaged in specified behaviors (e.g., eating, grooming, horizontal or vertical activity, awake but not moving, and sleeping) per condition (i.e., individual animals not rated on behavior). Chi-squares were used to determine the effect of stress on types of specific home cage behaviors between strains and diet conditions.

Locomotor Open Field (OF). The last OF measurement taken prior to restraint exposure was considered the baseline measure (Baseline Day 8). The OF chamber provided several indices of animal activity (e.g., horizontal activity, vertical activity). Baseline levels of horizontal and vertical activity were first analyzed with separate univariate ANOVAs. ANCOVAs were performed for the Stress Phase (Stress Day 6) and Post Stress (Post-stress Day 5) on baseline activity (Baseline Day 8) as a covariate to control for these differences.

Acoustic startle response (ASR) with and without pre-pulse. All animals were exposed to ASR/PPI on nine separate testing sessions (three during acclimation, one during Baseline Phase, three during Stress Phase, two during Post-stress Phase). Because of some problems with testing equipment (i.e., one chamber providing inconsistent values and data not recording), valid and reliable data were only available for the sessions Baseline Day 6, Stress Day 15, Post-stress Day 2. The data were examined for outliers. The group mean was used to

determine outliers (e.g., values 3 standard deviations below or above the group mean) (see descriptives in Appendix A). Significant global MANOVA results determined whether to examine subgroups separately. Global MANOVAs were conducted on startle amplitudes and percent of pre-pulse inhibition (%PPI) at each time point. Separate univariate ANOVAs were first performed on startle amplitude (ASR) and percent of pre-pulse inhibition (%PPI) to determine if there were any baseline differences between strains on these measures. If the ANOVA results revealed significant differences, then ANCOVAs for each ASR measure were performed using baseline values as covariates. To simplify data analysis, presentation, and interpretation, percent pre-pulse inhibition responses were collapsed across startle and pre-pulse stimulus intensities.

BIOLOGICAL DEPENDENT VARIABLES

Body weight

The appendix provides descriptives and statistical analyses for biological dependent variables. Figures 2-3 show the body weight data during each phase.

Baseline. Zucker rats weighed more than did Sprague-Dawley rats during the Baseline Phase [$F(1, 36) = 455.934$, $p < 0.05$]. The Strain X Diet interaction indicated that Zuckers fed a cafeteria diet weighed more than did all other groups [$F(1, 36) = 7.042$, $p < 0.05$]. There were no significant interactions or differences within each strain in body weight.

Stress Phase. Zuckers weighed more than did Sprague-Dawleys during the Stress Phase [$F(1, 36) = 470.419$, $p < 0.05$]. When baseline body weight was used as a covariate, there were no differences between strains in body weight during the Stress Phase. Subgroup analyses indicated that Zuckers fed bland chow only weighed less than did Zuckers fed a cafeteria diet during the Stress Phase [$t(18) = -2.221$, $p < 0.05$]. There were no significant interactions or differences in body weight within Sprague-Dawleys.

Post-stress Phase. Zucker rats weighed more than did Sprague-Dawley rats during the Post-stress Phase [$F(1, 36) = 492.148$, $p < 0.05$]. The Strain X Diet interaction indicated that Zuckers fed a cafeteria diet weighed more than did all other groups [$F(1, 36) = 4.923$, $p < 0.05$]. When baseline body weight was used as a covariate, Zuckers weighed more than did Sprague-Dawleys [$F(1, 35) = 4.471$, $p < 0.05$] and rats fed cafeteria food weighed more than did rats fed bland chow only [$F(1, 35) = 6.462$, $p < 0.05$], but the Strain X Diet interaction disappeared. Subgroup

comparisons indicated that Zuckers fed a cafeteria diet weighed more than did Zuckers fed bland chow only [t (18) = -2.416, p < 0.05]. There were no significant interactions or differences in body weight within Sprague-Dawleys.

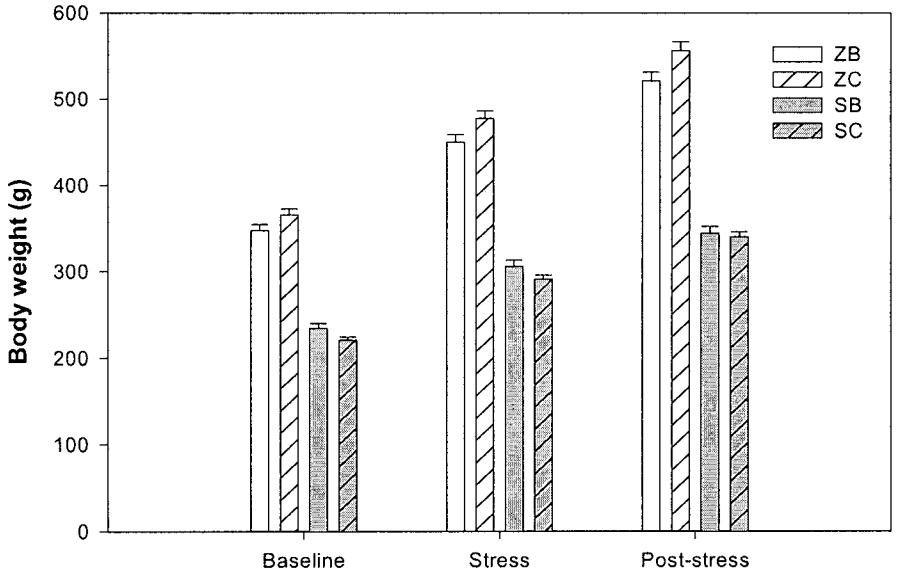

Figure 2. Body weight for each phase.

Repeated measures on Stress Days 3-17. Zuckers gained more weight than did Sprague-Dawleys within the Stress Phase [F (1, 36) = 482.003, p < 0.05]. Zuckers fed cafeteria food gained more weight than all other groups within the Stress Phase [F (7, 252) = 7.119, p < 0.05]. Body weight increased within the Stress Phase [F (7, 252) = 1954.317, p < 0.05]. When baseline body weight was used as a covariate, there were no differences between groups in how much body weight was gained within the Stress Phase. There was no main effect of Time or significant interactions with Time.

Repeated measures on Post-stress Days 3-14. Zucker rats gained more body weight than did Sprague-Dawleys within the Post-stress Phase [F (1, 36) =

501.063, $p < 0.05$]. Zuckers fed a cafeteria diet gained more weight than did all other groups [$F (1, 36) = 4.937$, $p < 0.05$]. Body weight increased within the Post-stress Phase [$F (5, 180) = 450.295$, $p < 0.05$]. The Time X Strain interaction revealed that Zuckers gained more weight than all other groups within the Post-stress Phase [$F (5, 180) = 16.416$, $p < 0.05$]. When baseline body weight was used as a covariate, Zuckers gained more weight than did Sprague-Dawleys [$F (1, 35) = 4.670$, $p < 0.05$]. Rats fed a cafeteria diet gained more weight than did rats fed bland food only from the Stress to Post-stress Phase [$F (1, 35) = 4.951$, $p < 0.05$].

Repeated measures on Two-day Period for Stress and Post-stress Phases. Body weight increased over each phase [$F (2, 72) = 2924.287$, $p < 0.05$]. The Time X Strain interaction revealed that Zuckers weighed more than Sprague-Dawleys at each phase [$F (2, 72) = 151.460$, $p < 0.05$]. The Time X Diet interaction indicated that rats fed a cafeteria diet weighed more than all other groups at each phase [$F (2, 72) = 6.069$, $p < 0.05$]. When baseline body weight was used as a covariate, rats fed a cafeteria diet gained more weight than did rats fed bland chow only [$F (1, 35) = 4.401$, $p < 0.05$]. The Time x Diet interaction revealed that rats fed a cafeteria diet gained more weight than did rats fed bland food only from the Stress to the Post-stress Phase [$F (1, 35) = 8.600$, $p < 0.05$].

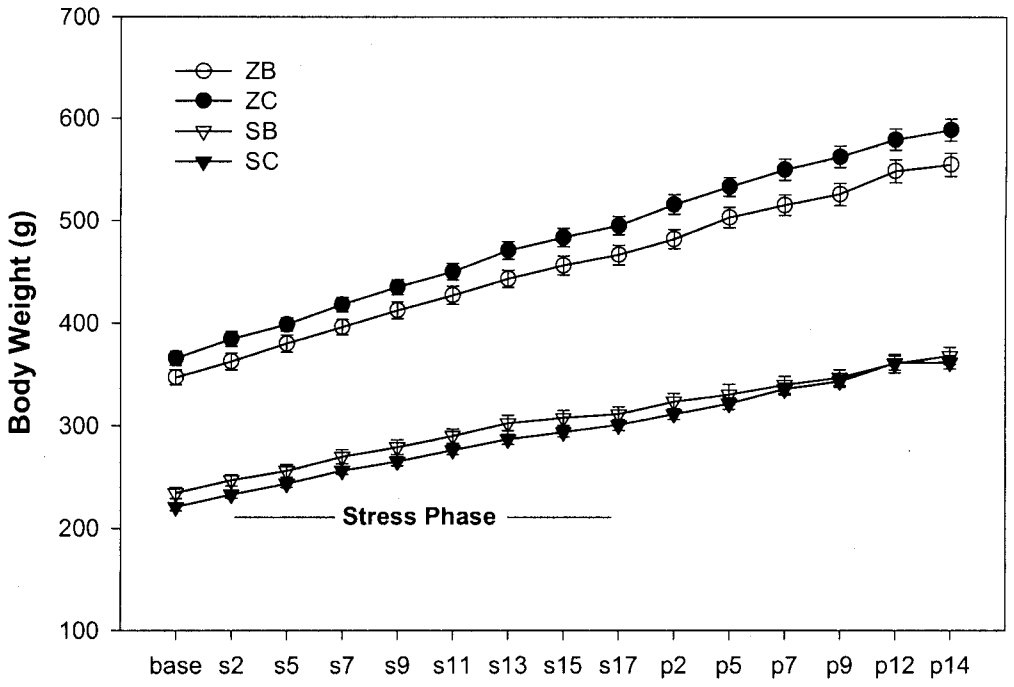

Figure 3. Body weight gain.

Within-subject changes in body weight gain between phases. All four groups (i.e., ZB, ZC, SB, SC) gained body weight from baseline to the Stress Phase and between the Stress Phase and Post-stress Phase (see appendix for detailed statistical presentation).

Lee Index

Zuckers had more body fat than did Sprague-Dawleys [$F(1, 36) = 480.213$, $p < 0.05$]. The Strain X Diet interaction revealed that Zuckers fed a cafeteria diet had more body fat than did all others [$F(1, 36) = 4.511$, $p < 0.05$]. Tukey HSD *post hoc* analyses revealed the following order for the amount of body fat among all groups (the presence of an equal sign between groups indicates non-significant differences): $ZC \geq ZB > SB > SC$. Zuckers fed a cafeteria diet had more body fat

than did Zuckers fed bland chow only [t (18) = - 2.164, p < 0.05]. There were no significant differences in body fat within Sprague-Dawleys.

Corticosterone

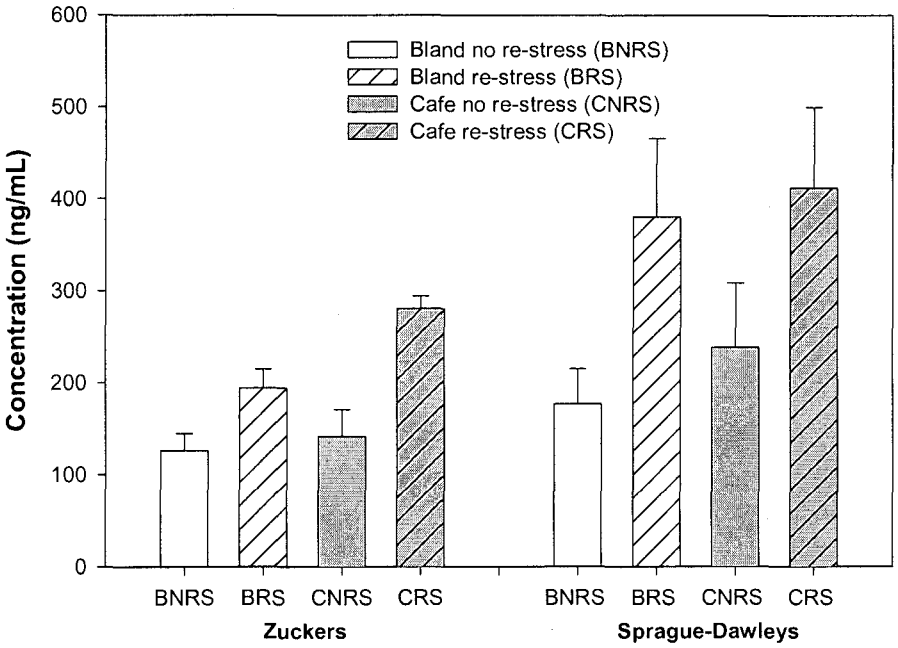

Figure 4. Corticosterone with and without re-exposure to restraint.

Figure 4 shows the corticosterone levels after re-exposure to stress. When analyses considered re-exposure to restraint as an additional independent variable along with Strain and Diet, Sprague-Dawleys [F (1, 31) = 9.359, p < 0.05] and rats re-exposed to restraint stress [F (1, 31) = 14.733, p < 0.05] had higher levels of corticosterone than did Zuckers and those rats that were not re-exposed to stress. Zuckers fed a cafeteria diet had greater corticosterone levels than did Zuckers fed bland chow only [F (1, 16) = 5.700, p < 0.05]. Zuckers re-exposed to stress had higher corticosterone levels than did Zuckers that were not re-exposed to restraint

[$F(1, 16) = 23.694$, $p < 0.05$]. There were no effects of diet within Sprague-Dawleys on corticosterone level in those rats that had been re-exposed to restraint.

Summary of Biological Data

The genetically obese Zucker rats weighed more and gained more weight than lean Sprague-Dawley rats at every phase of the experiment. Obese Zucker rats had significantly more body fat mass than lean Sprague-Dawley rats. Repeated acute restraint was effective in manipulating biological responses to stress. All animals increased body weight consistent with expected age-growth charts. All animals re-exposed to stress had increased corticosterone responses compared to animals not re-exposed to stress. In particular, obese Zuckers that were re-exposed to restraint prior to sacrifice had significantly greater levels of corticosterone than did obese Zucker rats not re-exposed to stress prior to sacrifice but these levels were blunted compared to lean Sprague-Dawley rat responses to stress. Corticosterone levels among Sprague-Dawleys had more variability than did Zuckers.

BEHAVIORAL DEPENDENT VARIABLES

Appendix A provides descriptives and statistical output for behavioral dependent variables. Figures 5 – 6 show the bland food consumption data during each phase.

Food consumption

Standard (or bland) chow

Baseline. Zuckers consumed more grams of standard chow than did Sprague-Dawleys at baseline [$F(1, 36) = 232.397$, $p < 0.05$]. Rats fed bland food only consumed more grams of bland chow than did rats fed cafeteria food [$F(1, 36) = 137.992$, $p < 0.05$]. The Strain X Diet interaction revealed that Zuckers fed bland chow only consumed more grams of bland food than did all other groups [$F(1, 36) = 4.588$, $p < 0.05$].

Stress Phase. Zuckers consumed more grams of bland chow than did Sprague-Dawleys during the Stress Phase [$F(1, 36) = 65.318$, $p < 0.05$]. Rats fed bland chow only consumed more bland chow than rats fed a cafeteria diet [$F(1, 36) = 155.941$, $p < 0.05$]. When baseline food consumption was used as a covariate, rats fed bland chow only consumed more grams of bland chow than did rats fed cafeteria food during the Stress Phase [$F(1, 35) = 12.190$, $p < 0.05$]. The Strain X Diet interaction revealed that Zuckers fed bland chow only consumed more bland chow than all others [$F(1, 35) = 8.957$, $p < 0.05$]. Tukey HSD *post hoc* analyses revealed the following order of significant differences ($p < 0.05$) in amount of bland chow consumed: ZB > SB > ZC > SC.

Post-stress Phase. Zuckers fed bland chow only consumed more grams of bland food than did all other groups [$F(1, 36) = 78.454$, $p < 0.05$]. Rats fed bland food only consumed more grams of bland food than did rats fed a cafeteria diet during Post-stress Phase [$F(1, 36) = 150.799$, $p < 0.05$]. When baseline food consumption was used as a covariate, rats fed bland food only consumed more grams of bland food than did rats fed a cafeteria diet during Post-stress Phase [$F(1, 35) = 10.184$, $p < 0.05$]. Tukey HSD *post hoc* analyses revealed the following order for the amount of bland chow consumed: ZB > SB > ZC > SC.

Repeated measures on Stress Days 3-17. Zuckers consumed more bland food than did Sprague-Dawleys within Stress Phase than did Sprague-Dawleys [$F(1, 36) = 143.011$, $p < 0.05$]. Rats fed cafeteria food consumed more bland food than did rats fed cafeteria food [$F(1, 36) = 251.554$, $p < 0.05$]. Bland food consumption decreased within Stress Phase [$F(7, 252) = 12.210$, $p < 0.05$]. The Time X Diet interaction revealed that rats fed a cafeteria diet consumed less bland chow than rats fed bland food only at all time points within the Stress Phase [$F(7, 252) = 3.052$, $p < 0.05$]. When baseline food consumption was used as a covariate, rats fed bland chow only consumed more bland chow within the Stress Phase than did rats fed a cafeteria diet [$F(1, 35) = 26.592$, $p < 0.05$]. The Strain X Diet interaction revealed that Zuckers fed bland chow only consumed more bland chow than did all other groups [$F(1, 35) = 5.775$, $p < 0.05$]. The Time X Strain interaction indicated that Zuckers consumed more bland chow than did all other groups at all points during the Stress Phase [$F(1, 35) = 3.250$, $p < 0.05$].

Repeated-measures analyses during Post-stress Phase Days 5-12. Zuckers consumed more bland chow than did all groups within the Post-stress Phase [$F(1, 36) = 113.363$, $p < 0.05$]. Rats fed bland chow only consumed more bland chow than did rats fed a cafeteria diet [$F(1, 36) = 141.785$, $p < 0.05$]. The Time X Diet interaction revealed that rats fed bland chow only consumed more bland food than did rats fed a cafeteria diet [$F(3, 108) = 6.967$, $p < 0.05$]. When baseline bland food consumption was used as a covariate, rats fed bland chow only consumed more bland chow than did rats fed a cafeteria diet [$F(1, 35) = 8.219$, $p < 0.05$]. The Time X Strain interaction revealed that Zuckers consumed more bland chow than did Sprague-Dawleys at all time points within the Post-stress Phase [$F(3, 105) = 3.687$, $p < 0.05$]. The Time X Strain X Diet interaction indicated that Zuckers fed bland chow only consumed more bland chow at all time points within the Post-stress Phase than did all other groups [$F(3, 105) = 3.029$, $p < 0.05$].

Repeated-measures analyses of Two-day periods during Stress and Post-stress Phases. Zuckers consumed more bland chow than did Sprague-Dawleys [$F(1, 36) = 85.615$, $p < 0.05$]. Rats fed bland chow only consumed more grams of bland food than did rats fed a cafeteria diet [$F(1, 36) = 184.380$, $p < 0.05$]. The Time X Strain X Diet interaction revealed that Zucker rats fed bland chow only consumed more bland chow during the Stress and Post-stress Phase than did all others [$F(1, 36) = 6.681$, $p < 0.05$]. When baseline bland food consumption was used as a covariate, rats fed bland chow only consumed more grams of bland food than did rats fed a cafeteria diet [$F(1, 35) = 14.497$, $p < 0.05$]. The Strain X Diet interaction indicated that Zuckers fed bland chow only consumed more grams of

bland chow and Sprague-Dawleys fed a cafeteria diet consumed fewer grams of bland chow than did all other groups [$F(1, 35) = 5.525$, $p < 0.05$]. Tukey HSD *post hoc* analyses revealed the following order for amount of bland chow consumed: ZB > ZC > SB > SC. The Time X Strain X Diet interaction revealed that bland chow consumption decreased the least for Sprague-Dawley rats fed a cafeteria diet [$F(1, 35) = 5.980$, $p < 0.05$].

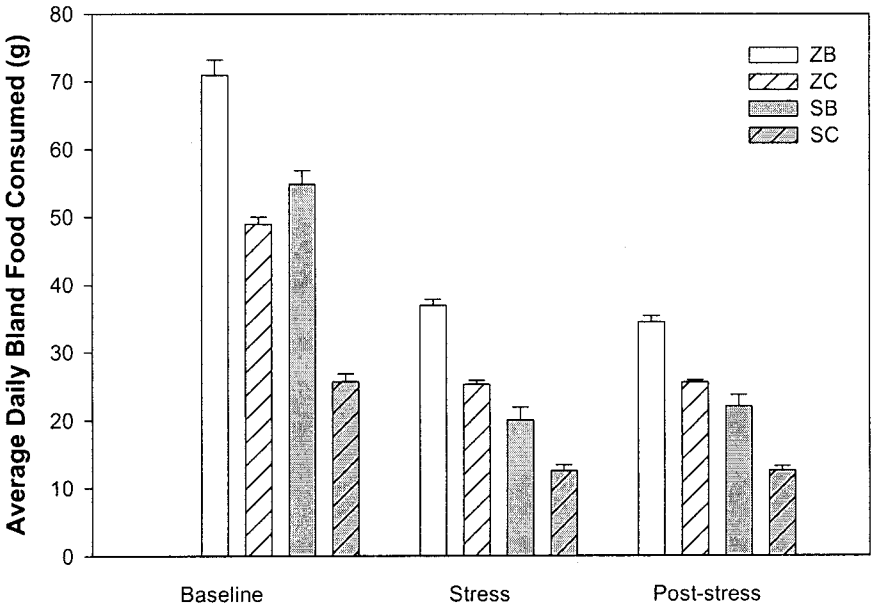

Figure 5. Average bland chow consumed at each phase.

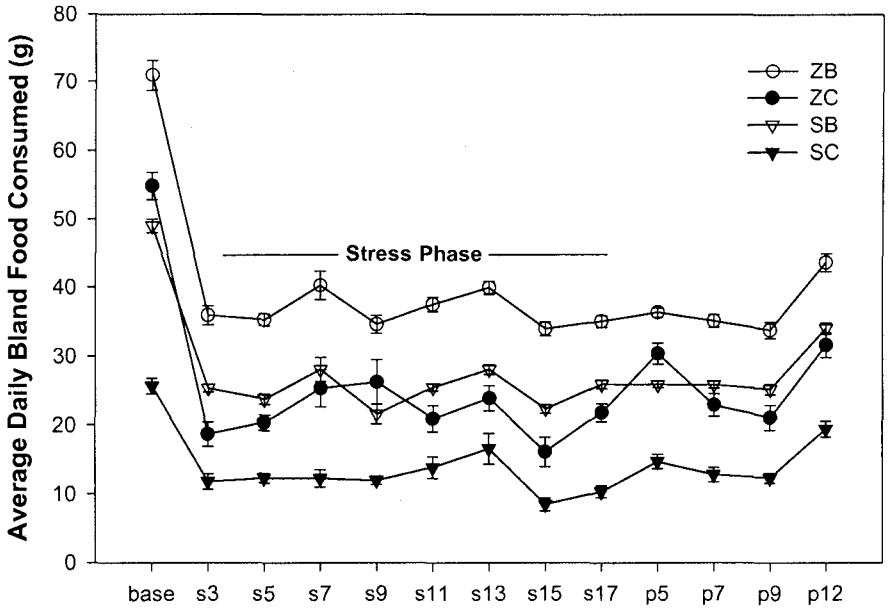

Figure 6. Average daily bland chow consumed by phase and day.

Lay's potato chips

Figures 7 and 8 show the amount of chips consumed.

Baseline. There were no differences at baseline between strains on the amount of chips consumed.

Stress Phase. Zuckers consumed more grams of chips than did Sprague-Dawleys during the Stress Phase [$F(1, 18) = 16.750$, $p < 0.05$].

Post-stress Phase. There were no differences between strains on the grams of chips consumed during Post-stress Phase.

Repeated measures on Two-day period during Baseline, Stress, and Post-stress Phases. The Time X Strain interaction revealed that chip consumption decreased among Sprague-Dawleys but increased among Zuckers during the Stress

Phase [F (2, 36) = 10.768, p < 0.05]. During Post-stress Phase, chips consumed decreased among Zuckers but increased slightly among Sprague-Dawleys.

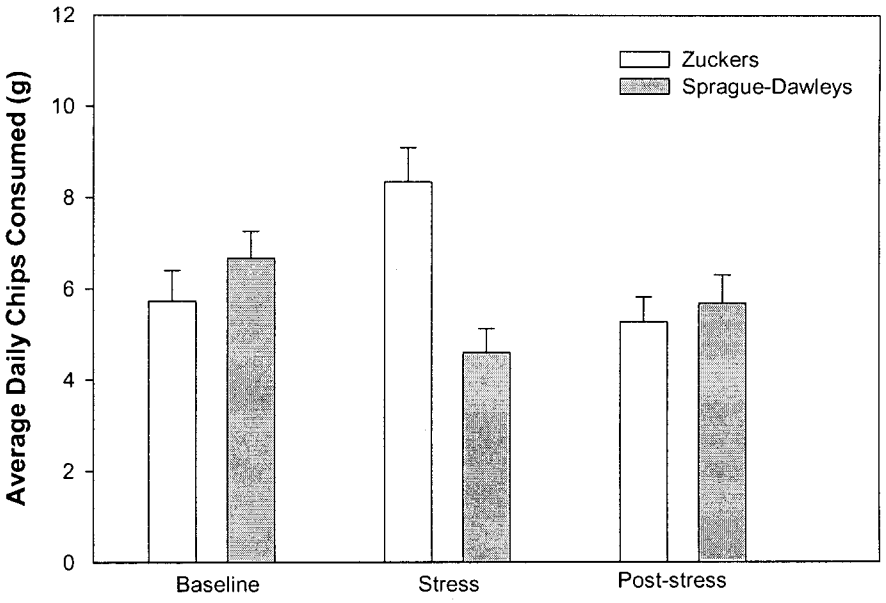

Figure 7. Average daily chips consumed each phase.

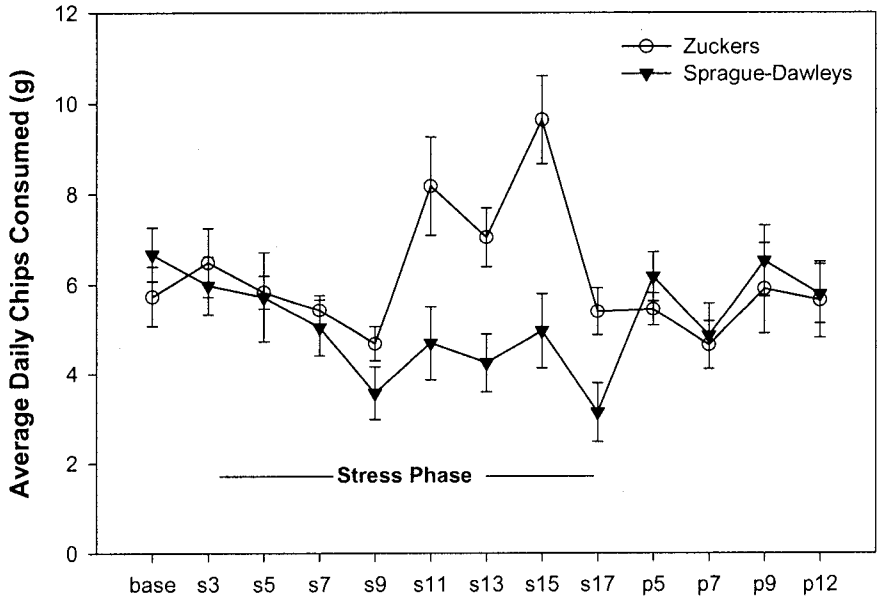

Figure 8. Average daily chips consumed by phase and day.

Oreo cookies

Figures 9 and 10 show the cookie consumption during Experiment I. Note that the significant decrease in cookie consumption on Post-stress Day 5 is attributed to measurement error. This day was not included in data analysis.

Baseline. There were no differences at baseline between strains on the amount of cookies consumed at baseline.

Stress Phase. Zuckers consumed more grams of cookies than did Sprague-Dawleys during Stress Phase [$F(1, 18) = 8.073$, $p < 0.05$].

Post-stress Phase. There were no differences between strains on the number of grams of cookies consumed during Post-stress Phase.

Repeated measures on Two-day period during Stress and Post-stress Phases. Zuckers consumed more grams of cookies than did Sprague-Dawleys [$F(1, 18) = 6.253$, $p < 0.05$]. Cookie consumption decreased during the Stress Phase and increased during the Post-stress Phase [$F(2, 36) = 21.004$, $p < 0.05$]. The Time X Strain interaction revealed that Sprague-Dawleys had a greater decrease during the Stress Phase and a greater increase during the Post-stress Phase in the number of grams of cookies consumed than did Zuckers [$F(2, 36) = 3.626$, $p < 0.05$].

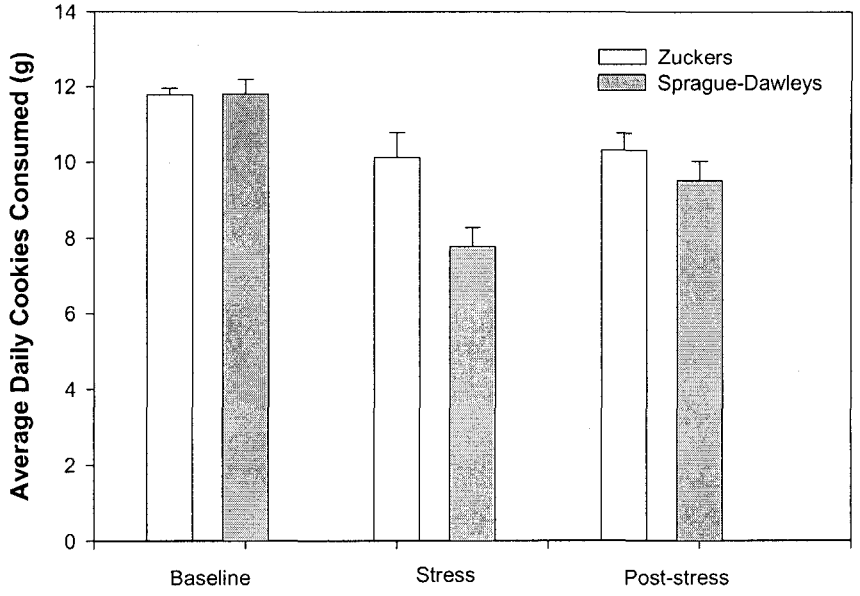

Figure 9. Average daily cookies consumed by phase.

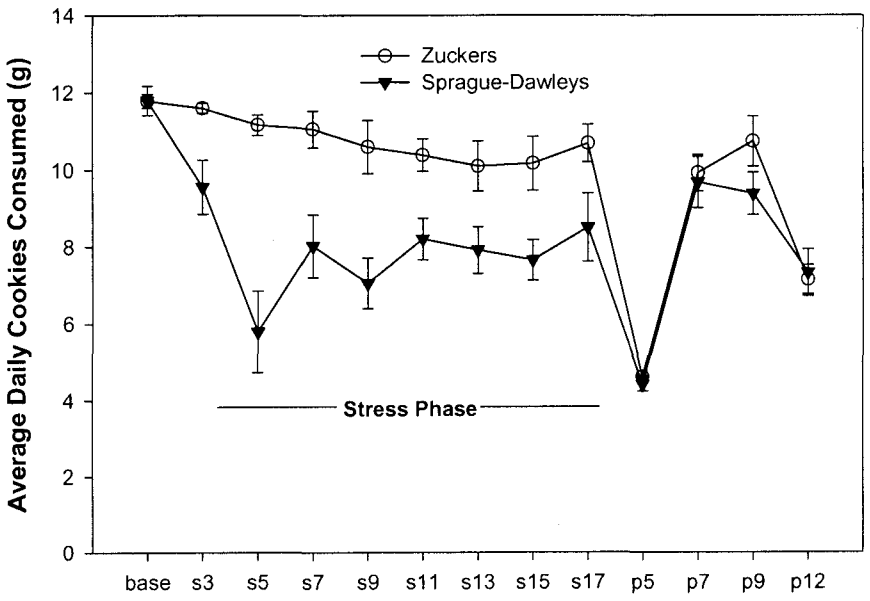

Figure 10. Average daily cookie consumption by phase and day.

Total Kilocalories Consumed

Figures 11 and 12 show the total amount of kilocalories consumed.

Baseline. Zuckers consumed more total calories than did Sprague-Dawleys [$F(1, 36) = 26.562$, $p < 0.05$]. Rats fed bland only food consumed more total calories than did rats fed a cafeteria diet [$F(1, 36) = 22.829$, $p < 0.05$]. The Strain X Diet interaction indicated that Zuckers fed bland chow only consumed more total kilocalories compared to all other groups [$F(1, 36) = 100.798$, $p < 0.05$].

Stress Phase. Zuckers consumed more total calories than did Sprague-Dawleys [$F(1, 36) = 169.969$, $p < 0.05$]. Rats fed bland only food consumed fewer total calories than did rats fed a cafeteria diet [$F(1, 36) = 95.738$, $p < 0.05$]. The Strain X Diet interaction indicated that Zuckers fed bland chow only consumed more total kilocalories compared to all other groups [$F(1, 36) = 7.796$, $p < 0.05$]. When total kilocalories were used as a covariate, Zuckers consumed more total calories than did Sprague-Dawleys [$F(1, 35) = 81.736$, $p < 0.05$]. Rats fed a cafeteria diet consumed more total kilocalories than did rats fed standard chow only [$F(1, 35) = 79.192$, $p < 0.05$]. The Strain X Diet interaction revealed that Zuckers fed a cafeteria diet consumed more total kilocalories than did any other group [$F(1, 35) = 8.508$, $p < 0.05$]. Tukey *post hoc* analyses revealed the following order of total kilocalories consumed across all groups: $ZC > ZB \geq SC > SB$.

Post-stress Phase. Zuckers consumed more total calories than did Sprague-Dawleys [$F(1, 36) = 59.157$, $p < 0.05$]. Rats fed bland only food consumed fewer total calories than did rats fed a cafeteria diet [$F(1, 36) = 112.390$, $p < 0.05$]. When baseline total kilocalories were used as a covariate, Zuckers

consumed more total kilocalories than did Sprague-Dawleys [$F(1, 35) = 22.674$, $p < 0.05$]. Rats fed a cafeteria diet consumed more total calories than did rats fed bland chow only [$F(1, 35) = 61.279$, $p < 0.05$].

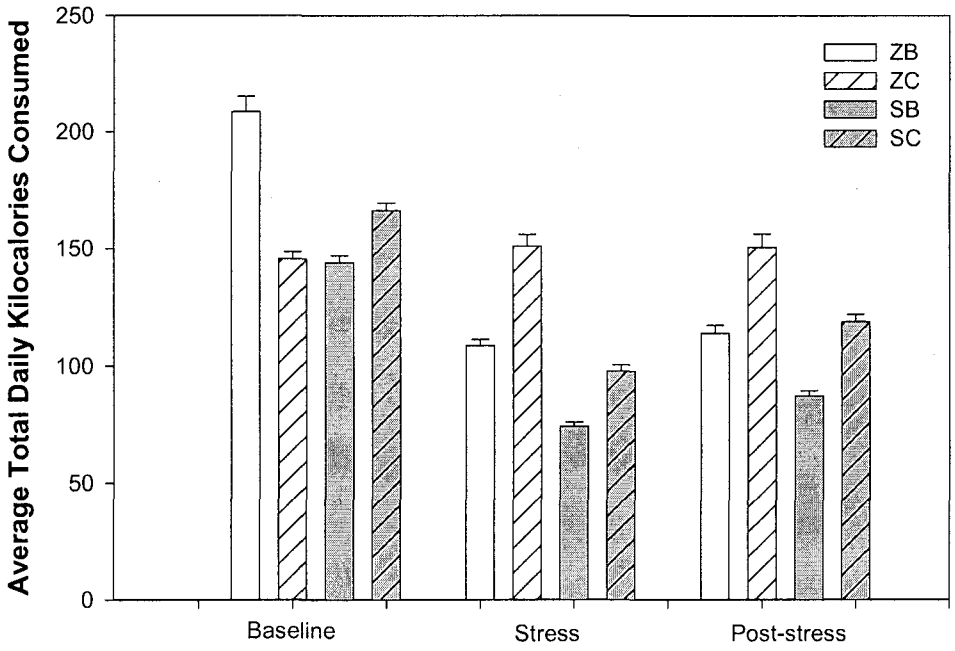

Figure 11. Average total kilocalories consumed at each phase.

Repeated-measures analyses on Total Kilocalories Consumed during Stress and Post-stress Phases. Zuckers consumed more total kilocalories than did Sprague-Dawleys [$F(1, 36) = 134.240$, $p < 0.05$]. Rats fed a cafeteria diet consumed more total kilocalories than did rats fed standard chow only [$F(1, 36) = 39.724$, $p < 0.05$]. The Strain X Diet interaction revealed that total kilocalories consumed remained constant among Zuckers fed a cafeteria diet and decreased among all other groups [$F(1, 36) = 15.501$, $p < 0.05$]. The Time X Strain interaction indicated that Zuckers consumed more total kilocalories at each phase than did Zuckers [$F(2, 72) = 12.439$, $p < 0.05$]. The Time X Diet interaction indicated that

rats fed cafeteria food consumed more total kilocalories during the Stress and Post-stress Phases than did rats fed bland chow only [$F (2, 72) = 101.342$, $p < 0.05$]. The Time X Strain X Diet interaction indicated that total kilocalories decreased for all groups except Zucker rats fed cafeteria food during the Stress and Post-stress Phases [$F (2, 72) = 76.099$, $p < 0.05$]. When baseline total kilocalories was used as a covariate, Zuckers consumed more total kilocalories than did Sprague-Dawleys [$F (1, 35) = 72.952$, $p < 0.05$]. Rats fed a cafeteria diet consumed more total kilocalories than did rats fed standard chow only [$F (1, 35) = 120.712$, $p < 0.05$]. The Strain X Diet interaction revealed that total kilocalories consumed remained constant among Zuckers fed a cafeteria diet and decreased among all other groups [$F (1, 35) = 6.145$, $p < 0.05$]. Tukey HSD *post hoc* analyses revealed the following order for amount of total kilocalories consumed: ZC > ZB $\geq$ SC > SB. The Time X Strain interaction indicated that Sprague-Dawleys had a greater decrease in total kilocalories during the Stress Phase than did Zuckers [$F (1, 35) = 8.091$, $p < 0.05$].

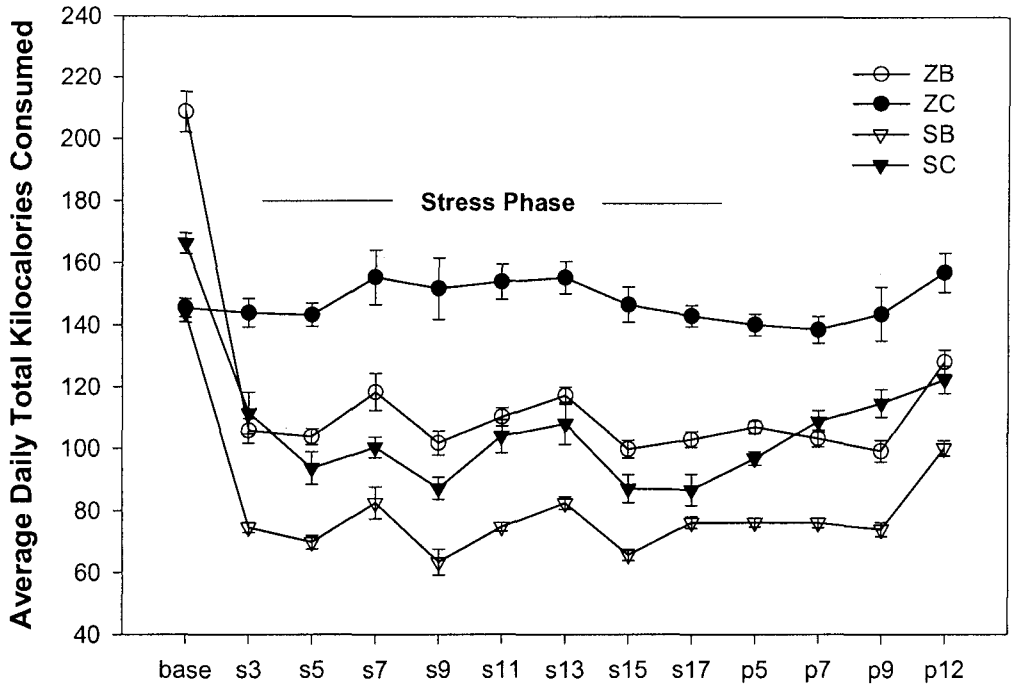

Figure 12. Average total number of kilocalories consumed by phase and day.

Summary of food consumption findings. Stress affected the amount of food and types of food eaten. Specifically, stress decreased the grams of standard chow consumed in lean and obese rats. The amount of standard chow consumption did not return to baseline levels once the stressor terminated or by the time the study ended. Chip consumption increased among obese rats, but decreased among lean rats during Stress Phase. Once the stressor terminated, chip consumption increased slightly among lean rats but decreased among obese rats. Cookie consumption decreased among lean rats but remained about the same among obese rats. Cookie consumption increased among lean rats but remained the same among obese rats once the stressor terminated. Stress decreased the total amount of kilocalories consumed for lean Sprague-Dawleys fed bland chow only and

cafeteria diet and obese Zuckers fed bland chow only. Importantly, stress did not affect the total amount of kilocalories consumed among obese Zuckers fed a cafeteria diet. Stress clearly shifted food preferences among obese Zuckers fed a cafeteria diet to salty foods while holding the total amount of kilocalories constant. Overall, unlike the other groups, obese Zuckers fed a cafeteria diet did not adjust their total food consumption in response to stress. Given that stressors are known to mobilize energy resources via both the sympathetic nervous system and HPA axis actions, this lack of adjustment is noteworthy.

Physical Activity

Home Cage Activity (HCA) Level during Baseline, Stress, and Post-stress Phases. Figure 13 below shows the level of home cage activity. There were no differences between groups in level of home cage activity at baseline. Sprague-Dawleys had a greater level of activity than did Zuckers during the Stress [$F(1, 36) = 40.096$, $p < 0.05$] and Post-stress Phases [$F(1, 36) = 123.725$, $p < 0.05$].

Repeated-measures analyses on HCA Level during all phases. Sprague-Dawleys had greater levels of home cage activity than did Zuckers [$F(1, 36) = 78.358$, $p < 0.05$]. The Time X Strain interaction indicated that Zuckers decreased the amount of home cage activity during the Stress Phase and Sprague-Dawleys increased the amount of activity during the Post-stress Phase [$F(2, 72) = 9.693$, $p < 0.05$].

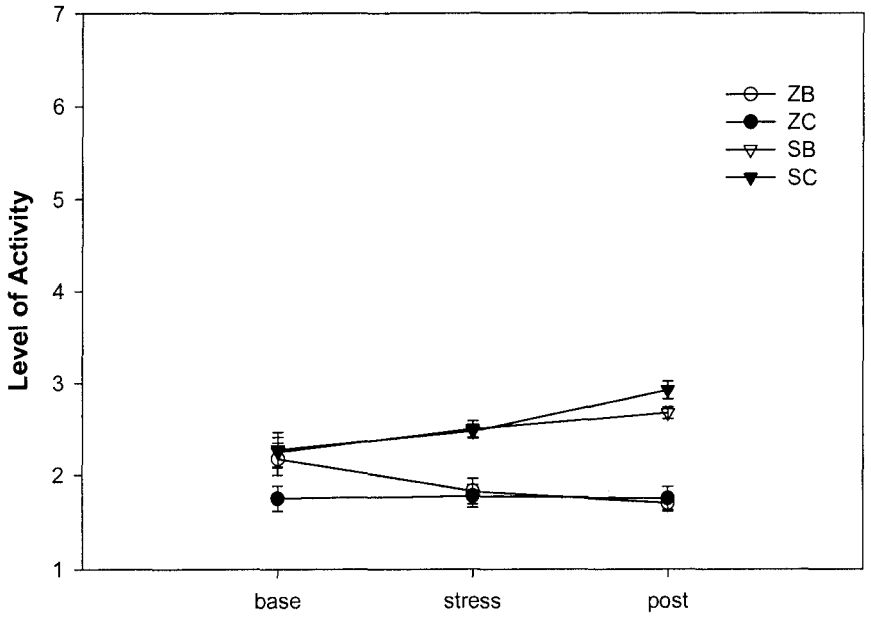

Figure 13. Level of activity in home cage.

Home Cage Behaviors. Baseline. Sprague-Dawleys engaged in more grooming behaviors in the home cage than did Zuckers [χ^2 (1) = 5.503, $p < 0.05$]. There were no differences in home cage behaviors based on diet at baseline.

Home Cage Behaviors. Stress Phase. Zuckers were more likely to be awake, but not moving, in the home cage than were Sprague-Dawleys [χ^2 (1) = 9.408, $p < 0.05$]. There were differences in home cage behaviors based on diet.

Home Cage Behaviors. Post-stress Phase. Zuckers were more likely to be sedentary (awake not moving) [χ^2 (1) = 11.815, $p < 0.05$] and sleeping [χ^2 (1) = 4.923, $p < 0.05$] in the home cage than were Sprague-Dawleys. Sprague-Dawleys engaged in more vertical activity in the home cage than did Zuckers [χ^2 (1) = 12.347, $p < 0.05$]. There were no differences in home cage behaviors based on diet during the Post-stress Phase.

Open Field (OF) Locomotor

Figures 14 – 15 show the locomotor activity between sessions for all phases.

Baseline. Zuckers had lower levels of horizontal activity [F (1, 36) = 90.598, $p < 0.05$] and vertical activity [F (1, 36) = 54.541, $p < 0.05$] than did Sprague-Dawleys. There were no within strain differences in locomotor activity based on diet during the Baseline Phase.

Stress Phase. Zuckers had lower levels of horizontal activity [F (1, 35) = 5.725, $p < 0.05$] than did Sprague-Dawleys. There were no within strain differences in locomotor activity based on diet during the Stress Phase.

Post-stress Phase. Zucker rats had lower horizontal activity [F (1, 35) = 35.175, $p < 0.05$] and vertical activity [F (1, 35) = 14.968, $p < 0.05$] than did

Sprague-Dawleys. There were no within strain differences in locomotor activity based on diet during the Post-stress Phase.

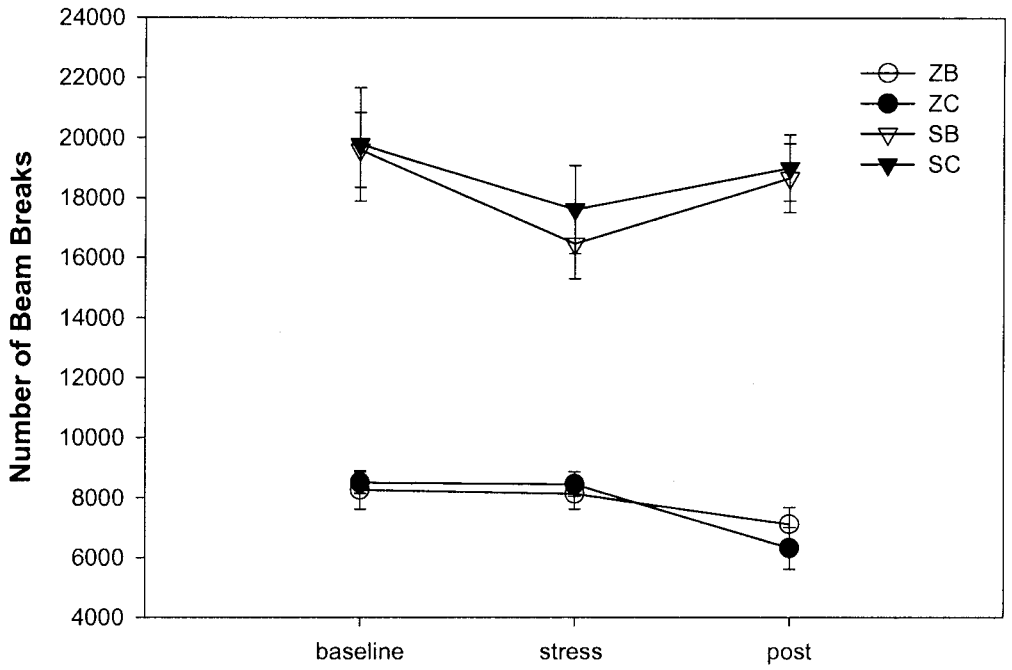

Figure 14. Horizontal activity in open field chamber.

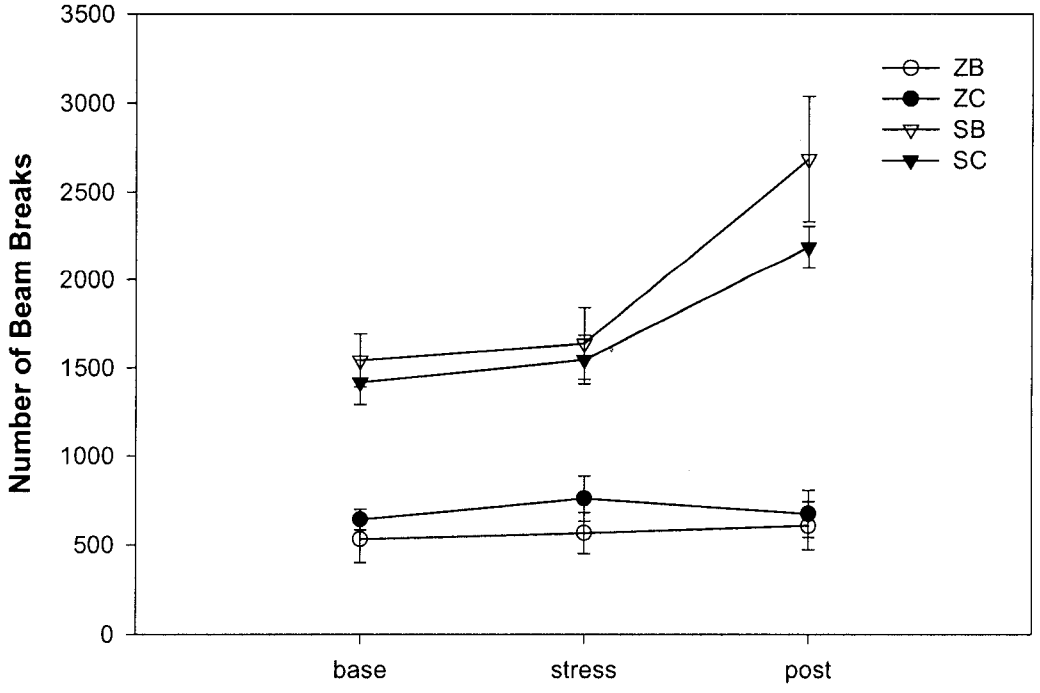

Figure 15. Vertical activity in open field chamber.

Repeated-measures analyses for Baseline, Stress Phase, and Post-stress Phase. Sprague-Dawleys had greater levels of horizontal activity [$F(1, 35) = 27.606$, $p < 0.05$] and vertical activity [$F(1, 35) = 14.328$, $p < 0.05$] than did Zuckers. The Time X Strain interaction revealed that the level of horizontal activity decreased during the Stress Phase among Sprague-Dawleys [$F(1, 35) = 9.470$, $p < 0.05$]. The Time X Strain interaction also revealed that horizontal activity increased during the Post-stress Phase among Sprague-Dawleys, but decreased among Zuckers. The Time X Strain interaction indicated that vertical activity increased during Post-stress Phase among Sprague-Dawleys [$F(1, 35) = 6.366$, $p < 0.05$].

Summary of within-subject changes for physical activity. Home cage activity increased among Sprague-Dawleys fed a cafeteria diet from Stress Phase to Post-stress Phase. Horizontal activity decreased from Baseline to Stress Phase among Sprague-Dawleys fed bland chow only. Vertical activity in the open field increased among Sprague-Dawleys from Stress Phase to Post-stress Phase.

Summary of physical activity findings. Sprague Dawleys had greater levels of home cage activity; Zuckers were more likely to be engaged in sedentary behavior, such as awake but not moving, than Sprague-Dawley rats. Obese rats had lower horizontal activity and vertical activity in the OF than lean rats during all phases, and these activity levels remained at nearly the same low level during all phases. Stress decreased horizontal activity among lean rats, but activity levels increased once the stressor terminated among lean rats. Interestingly, stress affected physical activity among lean rats not among obese rats.

COGNITIVE DEPENDENT VARIABLES

The appendix provides descriptives and statistical analyses for the behavioral dependent variables. Figures 16-19 show the startle responses and percent pre-pulse for each phase.

Acoustic Startle Response

Baseline. There were no differences between strains or diet condition on startle response or percent of pre-pulse inhibition (%PPI).

Stress Phase. Startle Amplitude and Percent of Pre-pulse inhibition (%PPI). Sprague-Dawleys had a greater startle response to 110 dB [$F(1, 36) = 21.140$, $p < 0.05$] and to 120 dB [$F(1, 36) = 18.492$, $p < 0.05$] than did Zuckers. Rats fed bland chow only had greater %PPI than did rats fed cafeteria food [$F(1, 36) = 7.541$, $p < 0.05$].

Post-stress Phase. Startle Amplitude and Percent Pre-pulse (%PPI). Sprague-Dawleys had a greater startle response to 110 dB stimulus [$F(1, 36) = 8.175$, $p < 0.05$] and to 120 dB stimulus [$F(1, 36) = 33.133$, $p < 0.05$] than did Zuckers. Rats fed bland chow only had greater %PPI than did rats fed cafeteria food [$F(1, 36) = 9.107$, $p < 0.05$].

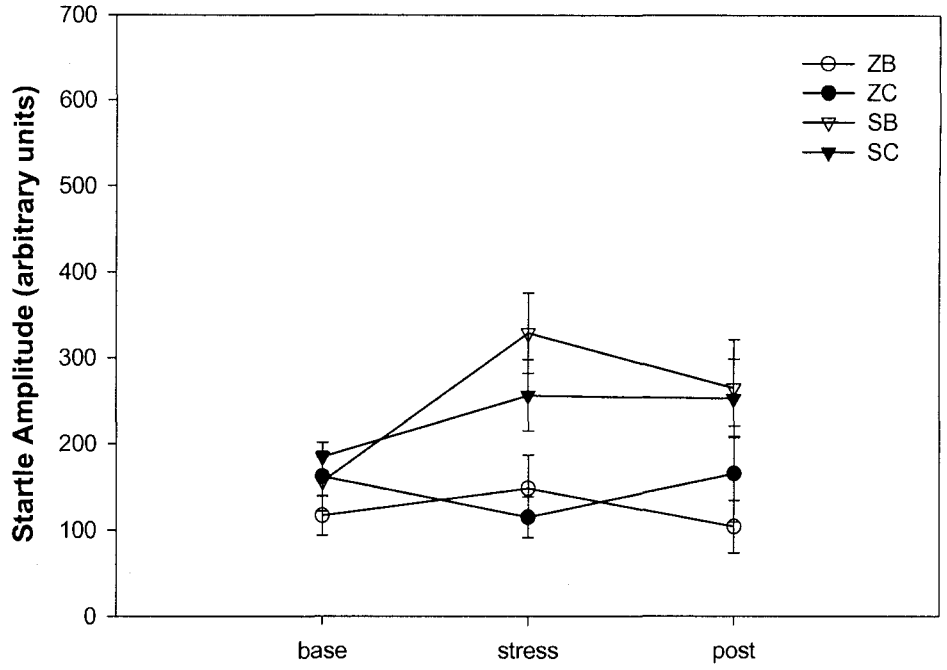

Figure 16. Startle amplitude to 110 dB stimulus.

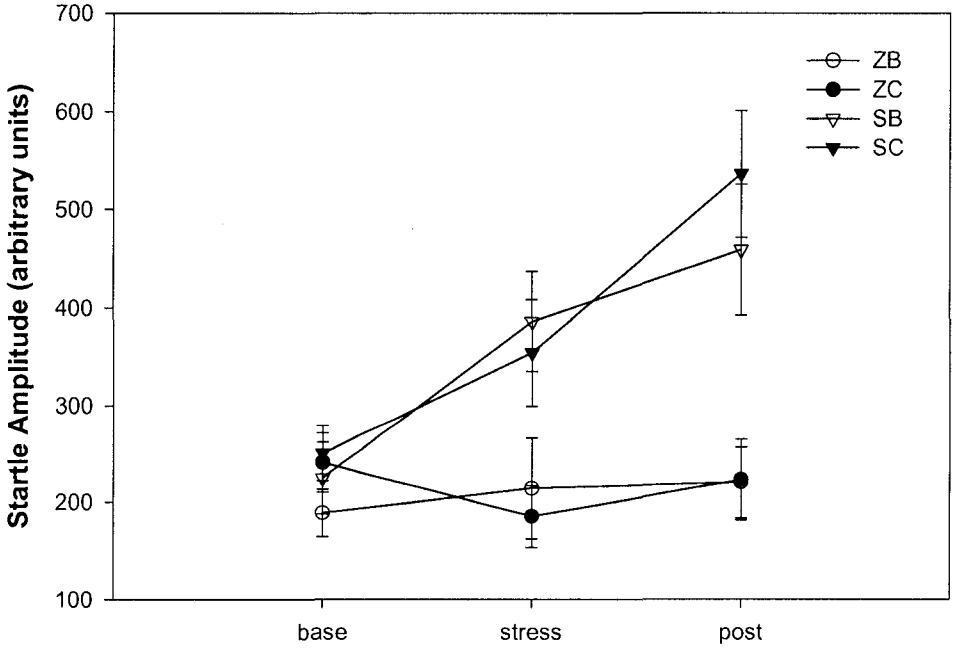

Figure 17. Startle amplitude to 120 dB stimulus.

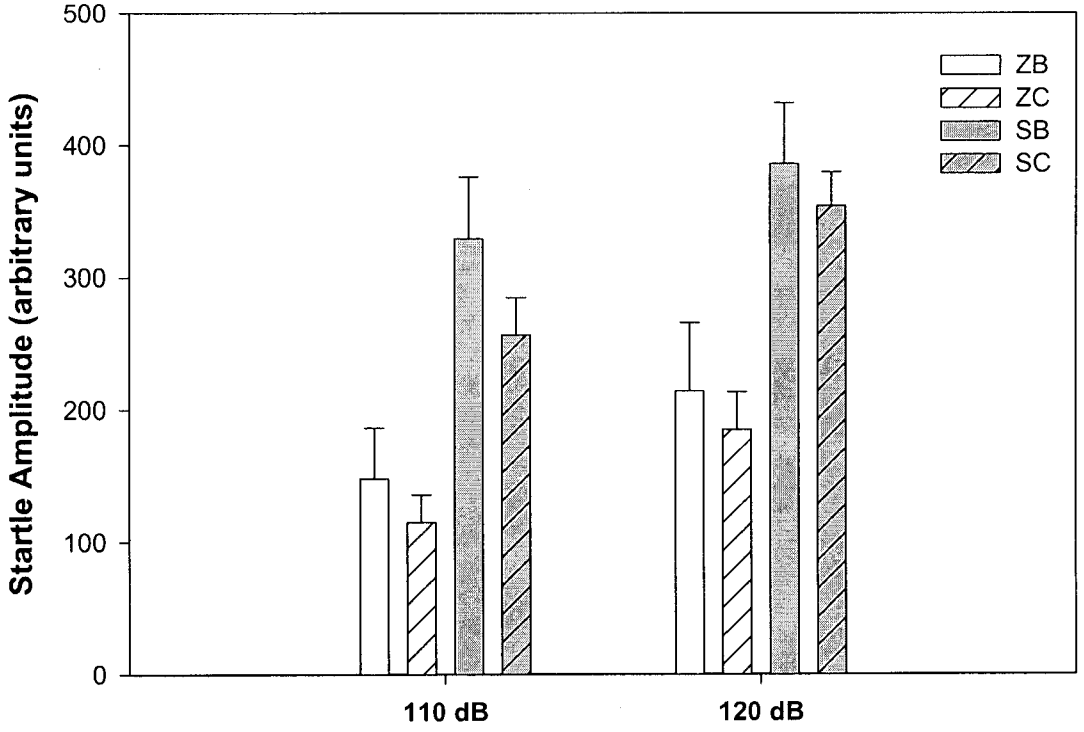

Figure 18. Startle amplitude for 110 dB and 120 dB during Stress Phase.

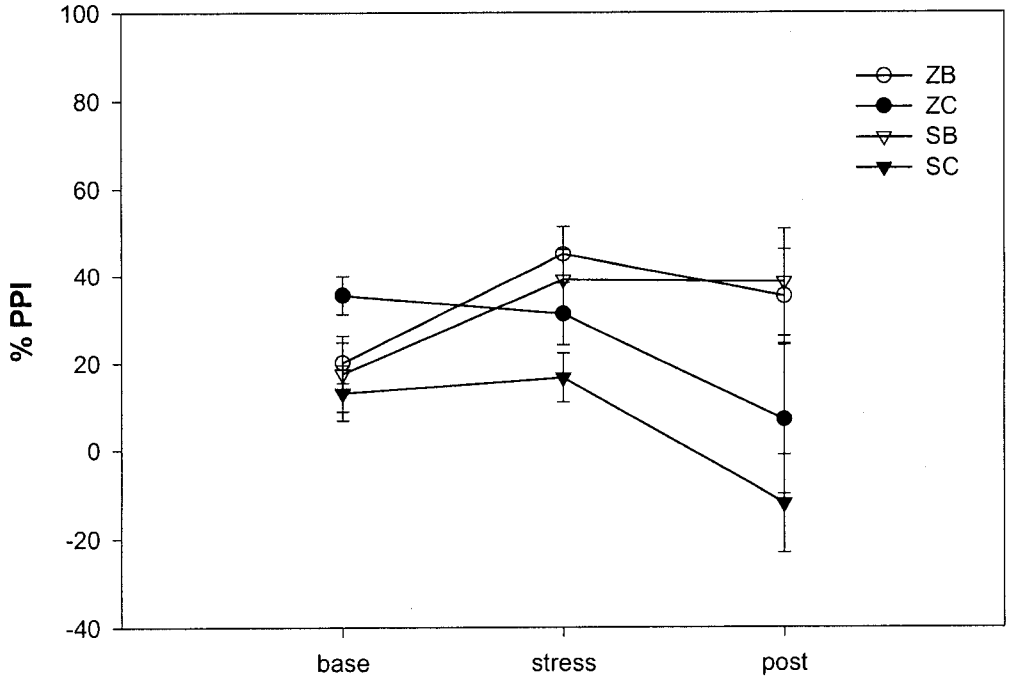

Figure 19. Average % PPI.

Repeated-measures analyses on startle amplitude through all phases. Sprague-Dawleys had greater startle to 110 dB [$F(1, 36) = 22.995$, $p < 0.05$] and 120 dB [$F(1, 36) = 30.978$, $p < 0.05$] than did Zuckers. Startle responses to 110 dB [$F(2, 72) = 3.282$, $p < 0.05$] and to 120 dB [$F(2, 72) = 15.796$, $p < 0.05$] increased during the Stress Phase. The Time X Strain interaction indicated that Sprague-Dawleys had a greater increase in startle to 110 dB during Stress Phase [$F(2, 72) = 4.184$, $p < 0.05$] and to 120 dB [$F(2, 72) = 14.245$, $p < 0.05$] during Stress and Post-stress Phases compared to Zuckers.

Repeated-measures analyses on %PPI through all phases. Rats fed cafeteria food had lower %PPI than did rats fed bland chow only [$F(1, 36) = 10.359$, $p < 0.05$]. Percent pre-pulse inhibition increased during Stress Phase and decreased during Post-stress Phase [$F(2, 72) = 3.195$, $p < 0.05$]. The Time X Diet interaction indicated that %PPI decreased among rats fed cafeteria food during the Post-stress Phase [$F(2, 72) = 6.085$, $p < 0.05$].

Within-subject changes for ASR and %PPI. Sprague-Dawleys fed bland chow only increased startle to 110 dB [$t(9) = -3.156$, $p < 0.05$] and 120 dB [$t(9) = -3.926$, $p < 0.05$] from Baseline to Stress Phase. Sprague-Dawleys fed a cafeteria diet increased startle to 110 dB [$t(9) = -2.624$, $p < 0.05$] and 120 dB [$t(9) = -4.032$, $p < 0.05$] from Baseline to Stress Phase. Sprague-Dawleys fed a cafeteria diet increased startle to 120 dB from Stress Phase to Post-stress Phase [$t(9) = -2.763$, $p < 0.05$]. Zuckers [$t(9) = -3.179$, $p < 0.05$] and Sprague-Dawleys [$t(9) = -2.317$, $p < 0.05$] fed bland chow only had an increase in %PPI from Baseline to Stress Phase.

Sprague-Dawleys fed cafeteria food decreased %PPI from Stress Phase to Post-stress Phase [$t(9) = -2.716$, $p < 0.05$].

Summary of ASR/PPI findings. The effect of stress on startle response and pre-pulse inhibition depended on Strain, Diet, and acoustic stimulus. During the Stress Phase all animals startled more to the 120 dB stimulus than to the 110 dB stimulus and startled less in the trial with the pre-pulse than in the trial without a pre-pulse. Sprague-Dawleys had greater startle responses during stress than did Zuckers, but there were no differences between strains in %PPI.

Stress-induced impairments in attentional processes were evident once the stressor terminated in animals fed the cafeteria diet but not bland chow. In particular, rats fed bland chow had a greater percent of pre-pulse inhibition than did rats fed a cafeteria diet during the Post-stress Phase. In animals fed the cafeteria diet, average pre-pulse inhibition was extremely low or even negative, reflecting an inability to process the information conveyed by the pre-pulse and/or reactivity to the pre-pulse itself – an abnormal cognitive response. The implication is that rats fed bland chow were able to attend to salient cues in the environment and were more resistant to the aftereffects of stress on attentional processes than were rats fed cafeteria food.

ASSESSMENT OF EXPERIMENT I HYPOTHESES

Hypothesis 1a. The genetically-induced model of obesity will produce rats heavier in body weight than will the diet-induced model of obesity.

Supported – Obese Zucker rats weighed significantly more at baseline and during the Post-stress Phase than did lean Sprague-Dawley rats. Obese Zucker rats were twice as heavy as lean Sprague-Dawley rats during the Stress and Post-stress Phases.

Hypothesis 1b. Genetically obese rats fed cafeteria food will weigh more than all other groups (ZC > ZB $\geq$ SC > SB).

Partially Supported – Zuckers fed a cafeteria diet were heavier than all other groups at baseline, but the Strain X Diet interaction was not significant during Stress and Post-stress Phases. Diet had a modest effect on body weight. The actual order of body weight during the Stress Phase and Post-stress Phases was as follows: ZC > ZB > SB $\geq$ SC.

Hypothesis 2a. Genetically obese rats will gain more weight and consume more food in response to stress compared to all other groups (ZC > ZB $\geq$ SC > SB).

Partially Supported – Obese Zucker rats gained significantly more body weight than did lean Sprague-Dawley rats during the Stress Phase when baseline body weight was not covaried. When the covariate was included in the analysis, this difference was not significant. Stress decreased the amount of standard chow consumed for both lean and obese animals. Zuckers fed bland chow only consumed more bland chow than did all other groups. The actual order of the amount of bland food consumed during the Stress Phase was as follows: ZB > SB

>ZC > SC. Obese Zucker rats consumed significantly more cookies and chips during the Stress Phase than did lean Sprague-Dawley rats. Obese Zucker rats fed a cafeteria diet also consumed a greater amount of total kilocalories than all other groups.

Hypothesis 2b. Stress will reduce physical activity to a greater extent among obese rats than lean rats (e.g., decreased horizontal activity, vertical activity, and decreased activity in the home cage) and will increase startle and decrease percent PPI more among obese rats than lean rats.

Partially supported – The findings in home cage support this hypothesis. As expected, obese rats had less home cage activity in response to stress. The level of home cage activity decreased among obese rats during Stress Phase and increased among lean rats during Post-stress Phase.

Obese rats were less active than lean rats at all phases. Horizontal activity decreased during the Stress Phase and increased during the Post-stress Phase among lean rats. Obese rats had no change in horizontal activity during the Stress Phase but had a slight decrease during the Post-stress Phase. Vertical activity in the OF among obese rats remained relatively low and unchanged in response to stress compared to a significant increase in among lean rats during the Post-stress Phase.

Lean rats had greater startle amplitudes but %PPI did not differ between lean and obese rats in response to stress. However, consumption of the cafeteria diet resulted in impaired percent PPI in both strains during the Post-stress Phase.

Hypothesis 2c. Obese rats will have a greater acute stress response indicated by higher corticosterone levels than others (ZC > ZB $\geq$ SC > SB).

Not supported – Lean Sprague-Dawley rats had greater corticosterone levels than did the obese Zucker rats (SC > SB > ZC > ZB).

DISCUSSION: EXPERIMENT I

One purpose of Experiment I was to identify the most feasible rodent model of obesity (genetic-induced or diet-induced obesity). The types of food (cookies and potato chips) to induce obesity were selected because they were calorically dense and highly palatable to rodents (Ely et al., 1997; Grunberg et al., 1988; Sclafani & Springer, 1976; Tomchesson, 2006) and humans. The genetically obese Zucker rats significantly weighed more and consumed more bland and cafeteria food than did the lean Sprague-Dawley rats.

Diet had a modest effect on body weight in comparison with the genetically obese Zucker. It is possible that the type of foods selected for the cafeteria diet were not calorically dense enough to produce the substantial weight gains reported genetic-induced rodent models of obesity. Previous experiments have used vegetable oil and shortening to produce substantial weight gains (Harris et al., 1998). Vegetable oil and shortening were purposely not chosen because of lack of generalizability, considering that humans are not likely to eat these foods in isolation. Because the amount of weight gain and food consumed was substantially greater in the genetically obese Zuckers, these rats were selected to examine further the effect of body type on stress responses in Experiment II. The findings in Experiment I may have been partially explained by differences in rat strain (Zucker vs. Sprague-Dawley). Therefore, Experiment II also addressed this potential limitation by using lean and obese rats from the same strain (Zucker).

The second purpose of Experiment I was to compare stress responses in obese and non-obese rats. The major effects of stress in Experiment I were: (1)

decreased bland food consumption in both strains; (2) altered cafeteria food consumption with increased chip consumption among Zuckers and decreased chip and cookie consumption among Sprague-Dawleys; (3) reduced overall kilocalorie consumption in all groups except for Zuckers fed the cafeteria diet; (4) greater corticosterone levels among lean rats re-exposed to stress, (5) greater levels of physical activity in familiar and novel environments among lean rats; and (6) greater startle responses among lean rats but impaired attentional processing among animals fed the cafeteria diet. These findings are discussed in more detail below.

Body weight and Feeding. Because all animals were young adults, it was expected that they would gain weight throughout all phases of the experiment. Obese Zuckers weighed more than lean Sprague-Dawleys at all phases of the experiment and gained more weight than Sprague-Dawleys when baseline body weight was not covaried. Once the stressor terminated, obese Zuckers gained more weight than did lean Sprague-Dawleys. Although all animals gained weight throughout the stress and non-stress phases, food consumption was altered. Specifically, during the Stress Phase lean rats decreased overall food consumption as well as consumption of bland food, chips, and cookies. In contrast, obese Zuckers fed the cafeteria diet did not alter overall kilocalorie consumption during the Stress Phase although they decreased bland food consumption, maintained cookie consumption at pre-stress levels, and increased chip consumption. This pattern is in contrast to Zuckers with access only to bland food – similar to Sprague-Dawleys, these animals decreased overall kilocalorie consumption and bland food consumption.

Lee Index. The Lee Index was included in Experiment I to determine whether the genetic-induced or diet-induced model produced the most robust rodent model of obesity. The genetic model of obesity produced Zuckers that had 36% more body fat than did Sprague-Dawleys compared to the diet-induced model that produced rats fed a cafeteria diet that had only 3% more body fat than rats fed bland chow only. During the Baseline Phase, it was clear that the genetic-induced model of obesity was the most feasible rodent model.

Corticosterone. In Experiment I, re-exposure to restraint stress resulted in higher levels of corticosterone compared to rats that had not been re-exposed to restraint stress, validating the restraint stressor in both lean and obese rats. Lean rats had higher levels of corticosterone than did obese rats and higher levels in response to stressor re-exposure than did obese rats. It appears from these data that obese rats may have less reactive HPA axis responses to stress than lean rats. Less reactivity to stress could be positive or negative. If the lower biological stress response meets the demands of the stressor while conserving the bodily resources, then less reactivity indicates an efficient stress response. However, if the lower biological stress response fails to mitigate the stressor and brain control centers (e.g., hypothalamus and hippocampus) continue to signal a threat, then less reactivity to stress is potentially health-harming over time.

The corticosterone findings should be interpreted with caution for several reasons. First, baseline corticosterone measurements were not obtained. Therefore, it is not known whether these differences in corticosterone levels were present at baseline. Experiment II addressed this limitation by adding a no stress

control condition for comparison of corticosterone in response to history of restraint stress. Second, the corticosterone measurements were taken 14 days after the Stress Phase ended. Although half of the animals in each condition were re-exposed to stress, it cannot be determined from this experiment whether the corticosterone level measured reflects the response to repeated acute restraint stress or the response to only the re-exposure.

Physical activity. Level of home cage activity decreased in response to stress among obese rats but increased among lean rats. In the open field, levels of general activity and exploratory behavior among obese rats were low and remained low at each phase of the experiment. The implication is that stress affects the level of physical activity in a familiar environment but does not affect activity and exploration in a novel environment among obese rats. Among lean rats, stress slightly decreased general activity in a novel environment (horizontal activity in OF) and slightly increased activity in a familiar environment (home cage).

Increased activity either in response to a stressor or once the stressor ceases can be interpreted as an adaptive behavioral change in response to stress. Sprague-Dawleys had greater amounts of horizontal plane and vertical activity than Zuckers across phases. During the Stress Phase, Sprague-Dawleys decreased horizontal activity and increased horizontal and vertical activity in the Post-stress Phase. In contrast, Zuckers had relatively little change in activity levels during the Stress Phase or Post-stress Phase. Overall, this pattern is suggestive of a more adaptive response among Sprague-Dawleys compared to Zuckers.

Home cage activity responses exhibit a similar pattern. Sprague-Dawleys, regardless of diet, have higher levels of home cage activity than Zuckers throughout the experiment, and home cage activity levels increased in the Post-stress phase compared to the Stress Phase. In contrast, Zucker home cage activity level decreased slightly during the Stress Phase and no change during the Post-stress Phase.

Acoustic startle response (ASR) with and without pre-pulse. Acoustic startle response (ASR) and pre-pulse inhibition (PPI) were used in the present project to index the effect of stress on cognitive processes (Acri, 1992, 1994; Acri et al., 1991; Faraday et al., 1999). When an increase in startle or no change in startle is accompanied by a decrease in the percent of pre-pulse inhibition, the interpretation is an impairment in attentional processing. Stress can affect the magnitude of startle responses and pre-pulse inhibition. Stress-induced impairments in attentional processing are marked by increased or no changes in startle accompanied by decreased pre-pulse inhibition (Acri, 1992; 1994; Faraday et al., 1999). Sprague-Dawley rats had a greater startle response to 110 dB and 120 dB than did obese Zucker rats during the Stress Phase. There were no strain differences in percent PPI. The major change in PPI was an effect of the cafeteria diet to reduce PPI during both the Stress and Post-stress phases.

Overall, these data indicated that the genetically-induced model of obesity was more suitable for use in Experiment II. In addition, these findings suggest that Zuckers may be less behaviorally and biologically responsive than Sprague-Dawleys to a repeated acute mild stressor.

OVERVIEW: EXPERIMENT II

The purpose of Experiment II was to use the genetic model of obesity to explore whether behavioral and biological stress responses differ in male and female rats by body type when background genetic strain is controlled by using only Zucker animals (i.e., subjects were genetically lean and obese rats from the Zucker strain). A major change from Experiment I was that only a bland food diet was used. The independent variables were Body Type (lean or obese), Sex (male or female), and Stress (stressed or unstressed). The behavioral dependent measures from Experiment I were used in Experiment II: (1) behaviors (feeding and activity); (2) cognitive processes (attention); and (3) biological (body weight, body fat, and corticosterone). The goal of using this particular combination of dependent measures was to characterize any differential stress response patterns that may exist between obese and non-obese male and female rats. The experiment was conducted in three experimental phases (Baseline, Stress, and Post-stress Phases) using a mixed design. Differences in the behavioral and biological responses to stress were examined by the between-subjects variables of Body type, Sex, Stress; within-subject variable Time; and the interaction of these variables.

On Experimental Day 1 (rats arrived at the animal facility), animals were randomly assigned to either the stressed or unstressed condition. The animals were subsequently assigned to one of two cohorts for logistical reasons. Each cohort consisted of 32 animals balanced across conditions. The biological and behavioral measures were given in the same order for each cohort. Experiment II was conducted using the procedures of Experiment I, but using male and female rats of

the same strain (i.e., Zucker rats) in stressed and unstressed conditions. The Baseline, Stress, and Post-stress Phases each lasted for 14 days. Animals assigned to the unstressed condition were exposed to the same behavioral measures as the stress condition but were not restrained at any point during the experiment. During the Stress Phase, animals assigned to the stress condition were exposed to 20 minutes of repeated acute restraint stress for 14 consecutive days. Table 74 in the appendix provides a timeline for the procedures that were used in Experiment II.

SPECIFIC AIMS AND HYPOTHESES: EXPERIMENT II

Experiment II examined behavioral and biological responses to stress in male and female, obese and non-obese Zucker rats. The central hypotheses were derived from Experiment I and from the existing literature on sex differences. These include: (1) obese rats will be less responsive behaviorally and biologically to stress compared to lean rats; (2) responses to stress will differ in males and females.

Specific Aim #1: Determine biobehavioral responses to stress in obese and non-obese rats

Hypothesis 1a. Obese rats and lean rats will decrease food consumption in response to stress but will continue to gain weight consistent with growth curves for each strain.

Rationale. Based on findings from Experiment I, stress decreased consumption of bland food in lean and obese animals while rates of body weight gain were maintained.

Hypothesis 1b. Obese rats will have a blunted corticosterone response compared to lean rats.

Rationale. Findings from Experiment I indicate that magnitude of corticosterone responses in animals re-exposed to stress and not re-exposed to stress was smaller in obese animals compared to lean animals.

Hypothesis 1c. Obese rats will have lower levels of physical activity overall compared to lean rats and will not alter physical activity responses when exposed to stress (e.g., horizontal activity, vertical activity, and home cage activity); lean rats will increase activity levels in response to stress.

Rationale. Findings from Experiment I indicated that obese Zuckers did not change activity behaviors in response to a repeated, acute stressor.

Hypothesis 1d. Stress will increase startle responses in lean animals compared to obese animals without altering percent PPI.

Rationale. Findings from Experiment I indicated that lean animals have greater startle responses than obese animals but that PPI does not change when animals have access only to bland food. Findings from the existing literature (Faraday, 2002) indicate that repeated restraint stress increases startle responses.

Specific Aim #2: *Determine the effect of sex on biobehavioral responses to repeated acute stress in obese and non-obese rats*

Hypothesis 2. Stress will have different effects on behavioral and biological responses of male and female, obese and non-obese rats.

Rationale. There is a limited literature that compares the responses of male and female Zucker lean and obese animals to stress, overall this literature suggests that there will be sex differences in these responses. To the author's knowledge, the only study that compared these four groups focused on corticosterone responses and found that obese Zucker males produced greater amounts of corticosterone in response to three different types of stressors (e.g., cold stress, immobilization, and ether) than did all other groups (e.g., lean Zucker males and females and obese Zucker females) (Guillaume-Gentil, 1990).

METHODS: EXPERIMENT II

SUBJECTS

A total of 64 obese and non-obese Zucker rats were used in Experiment II (32 males and 32 females). Obese Zucker rats (fa/fa) served as the model of obesity based on the findings of Experiment I. Lean Zucker rats (Fa/?) served as the non-obese rats to provide a more similar control group than would be provided by other strains of non-obese rats (e.g., Sprague-Dawleys). Bland food (Harlan Teklad) and water were continuously available. The rats were approximately 20 days old and weighed 40 - 50 grams at the beginning of the experiment.

HOUSING

Upon arrival, animals were randomly assigned to experimental conditions (stressed group or unstressed control group) balancing for Genotype and Sex. All cages were kept in a climate-controlled room maintained at approximately 23°C and 50% relative humidity. The room was maintained on a 12-hour reversed light-dark cycle (lights off at 0400 hours; lights on at 1600 hours). Animals were single-housed in standard polycarbonate rat cages (40 cm x 20 cm x 20 cm) with no additional objects or other animals.

Independent Variables

The between-subjects independent variables were Body type (fatty Zucker or lean Zucker), Sex (male or female), and Stress conditions (stressed or unstressed). The stress manipulation of 20-minute restraint was identical to the procedure used in Experiment I. Rats were stressed for 14 consecutive days.

Dependent Variables

The same dependent variables that were used in Experiment I were used in Experiment II: body weight, food consumption, physical activity (home cage and open field), indices of attention (acoustic startle response and pre-pulse inhibition), and a biochemical measure of the stress response (corticosterone). Table 74 in Appendix A provides a timeline for Experiment II.

Differences from Experiment I

Experiment II included several refinements based on the findings in Experiment I. First, to control background genetics, the same rat strain (Zucker) was used for lean and obese animals. Second, diet was not manipulated in Experiment II; standard bland chow was the only food available. Third, Experiment II included males and females and a between-subjects design in which rats were assigned to stressed and unstressed conditions.

Experimental Design and Sample Size

Experiment II was a 2 (Body type: obese or non-obese) X 2 (Sex: male or female) X 2 (Stress condition: stressed or unstressed) full factorial mixed design with between-subjects factors of Body type, Sex, and Stress condition and within-subject factor of Time. There were 8 subjects per cell for a total of 64 rats. However, one male unstressed fatty Zucker rat died 3 days before Experiment II ended. Autopsy results revealed that the rat had an enlarged heart and liver and a small amount of fluid in the chest. These symptoms are consistent with heart failure. The digestive tract of this animal was completely empty, suggesting that he had not eaten recently.

The data for this animal were excluded from Post-stress Day 8 (first day when food consumption stopped) through Post-stress Day 12 (end of experiment).

RESULTS: EXPERIMENT II

General Data Analytic Approach

The following abbreviations are used in reference to the eight conditions: lean males control (LMC), lean males stressed (LMS), obese males control (OMC), obese males stressed (OMS), lean females control (LFC), lean females stressed (LFS), obese females control (OFC), and obese females stressed (OFS). All effects and tests reported are significant at $p < 0.05$ unless otherwise noted. Because animals were run in two different cohorts that were staggered by one day for logistical purposes, preliminary analyses were performed on baseline values to ensure that cohorts did not differ. The cohorts were combined because there were no differences.

The following strategy was used to collapse the data for interpretation by phase. The last measurement taken prior to the start of restraint stress was used as baseline. Then, the mean was calculated and graphed for every measurement taken (see appendix for descriptives and figures). The graphs were examined for patterns of activity. Because there were consistent patterns of activity, the average of two specific time points were selected to represent the Stress Phase and Post-stress Phase for each dependent variable. The criteria used to select these two specific time points were as follows: (1) relatively close temporal proximity to when data collection occurred for all dependent variables during each phase (i.e., to control for effects of time and maturation and to allow comparison of activity across

measures), and (2) sufficient time elapse to capture the effects of stress and effects after the stressor terminated. All graphed data are group means and standard error of the mean. Only measures that had significant findings are reported.

Body weight. A total of 24 body weight measurements were taken. Because body weight differences were the primary variable of interest, body weight analyses were performed both with and without baseline values as covariates. This approach allowed the examination of data with and without controlling for initial body weight differences. The final body weight measurement taken prior to the start of restraint (Baseline Day 10) was used as a covariate. The body weight measurements taken on Stress Days 9 and 11 were averaged to represent the mean body weight during the Stress Phase. Separate univariate analyses of covariance (ANCOVAs) were conducted using the mean body weight to determine whether the groups differed in body weight during the Stress Phase. The body weight measurements taken on Post-stress Days 3 and 5 were averaged to represent the mean body weight during the Post-stress Phase. Repeated-measures ANCOVAs were conducted on body weight measurements through Stress Day 14 to determine whether stress affected body weight gain over time.

Food consumption. Because body weight is related to food consumption, and body weight was the primary variable of interest, food consumption analyses also were performed both with and without baseline values as covariates. This approach allowed the examination of data with and without controlling for initial feeding differences. A total of 18 food consumption measurements were taken. The final food consumption measurement taken prior to the start of restraint (Baseline

Day 10) was used as a covariate. Food consumption measurements taken on Stress Days 9 and 11 were averaged to represent the mean food consumption during the Stress Phase and analyses were conducted on the mean. Food consumption measurements taken on Post-stress Days 3 and 5 were averaged to represent the mean food consumption during the Post-stress Phase and analyses were conducted on the mean. Separate univariate analyses of covariance (ANCOVAs) were conducted using the mean food consumption from baseline to determine whether the groups differed in food consumption during the Stress and Post-stress Phases. Repeated-measures ANOVAs were conducted to determine the amount of food consumption changed over time.

Lee Index. Univariate analysis of variance (ANOVA) were performed to determine whether the groups differed in the amount of body fat.

Home Cage Activity. Four separate home cage activity ratings (conducted by two different raters) were made during each phase (Baseline, Stress, and Post-stress) for each animal. These four ratings were averaged together and the means represented the levels of home cage activity. Separate univariate ANCOVAs were used to detect differences between groups during the Stress and Post-stress Phases on home cage activity. To control for baseline differences home cage activity, ANCOVAs were performed for subsequent analyses of Stress and Post-stress Phases. Four separate observations (conducted by two different raters) tallied the number of animals engaged in specified behaviors (e.g., eating, grooming, horizontal or vertical activity, awake but not moving, and sleeping) per condition. was used to determine differences between groups in the number of animals

engaged in specific home cage behaviors. Chi-square was used to determine differences between groups in the number of animals engaged in specific home cage behaviors.

Locomotor Open Field. A total of six measurements in the open field chamber were taken. Locomotor data measured on Stress Days 9 (Cohort A) and 10 (Cohort B) were used in data analyses to represent the Stress Phase. Locomotor data measured on Post-stress Days 3 (Cohort A) and 4 (Cohort B) were used in data analyses to represent the Post-stress Phase. Separate univariate ANOVAs were first performed on horizontal and vertical activity to determine if there were any baseline differences between strains on these measures. If the ANOVA results revealed significant differences, then ANCOVAs for each activity measure were performed using baseline values as covariates.

Acoustic startle response with and without pre-pulse. A total of seven ASR measurements were taken (two during acclimation phase, one during baseline phase, two during the stress phase, and two during the Post-stress phase). Data collected during the acclimation phase were not used in the statistical analyses. All animals were first analyzed together. Significant global MANOVA results determined whether to examine subgroups separately. Global MANOVAs were conducted on startle amplitudes and percent of pre-pulse inhibition (%PPI) at each time point. Percent pre-pulse (%PPI) was calculated as [(amplitude of trial without pre-pulse) – (amplitude of trial with pre-pulse)/amplitude of trial without pre-pulse] X 100. The product was analyzed as % PPI. These calculations were based on established procedures (Acri 1994; Faraday & Grunberg, 2000). To simplify data

analysis, presentation, and interpretation, percent pre-pulse inhibition responses were collapsed across startle and pre-pulse stimulus intensities.

After two acclimation testing sessions, animals were exposed to a baseline measurement of ASR/PPI (Baseline Day 8). Separate univariate ANOVAs were first performed on startle amplitude (ASR) and percent of pre-pulse inhibition (%PPI) to determine if there were any baseline differences between strains on these measures. If the ANOVA results revealed significant differences, then ANCOVAs for each ASR measure were performed using baseline values as covariates. ASR/PPI data used in analyses for the Stress Phase were measured on Stress Days 11 and 12 and Post-stress Days 11 and 12. Data for one obese unstressed male rat were excluded because of poor health at the time of testing.

BIOLOGICAL DEPENDENT VARIABLES

Body weight

The appendix provides descriptives and statistical analyses for biological dependent variables. Figures 20 and 21 show the body weight data for all phases.

Baseline Phase. Obese [$F(1, 56) = 495.089$, $p < 0.05$] and male rats [$F(1, 56) = 40.003$, $p < 0.05$] weighed more than did lean and female rats at baseline. The Body Type X Sex interaction revealed that obese females weighed the most and lean females weighed the least at baseline [$F(1, 56) = 97.455$, $p < 0.05$]. Lean males weighed more than did lean females [$F(1, 28) = 192.985$, $p < 0.05$]. Obese females weighed more than did obese males [$F(1, 28) = 4.765$, $p < 0.05$]. There were no differences in body weight between subjects assigned to the stress and control groups within each body type.

Stress Phase. Obese [$F(1, 56) = 787.078$, $p < 0.05$] and male [$F(1, 56) = 86.078$, $p < 0.05$] rats weighed more than did lean and female rats during the Stress Phase. When body weight was used as a covariate, obese rats weighed more than did lean rats [$F(1, 55) = 30.592$, $p < 0.05$] and males weighed more than did females [$F(1, 55) = 59.904$, $p < 0.05$]. Lean males weighed more than did lean females [$F(1, 27) = 23.707$, $p < 0.05$]. Obese male rats weighed more than did obese female rats [$F(1, 27) = 18.809$, $p < 0.05$]. There were no differences in body weight between the stress and control groups within obese rats.

Post-stress Phase. Obese [$F(1, 56) = 901.343$, $p < 0.05$] and male [$F(1, 56) = 140.903$, $p < 0.05$] rats were heavier than lean and female rats during the Post-stress Phase. The Body Type X Sex interaction revealed that obese males

followed by obese females weighed more than all other groups [$F(1, 56) = 79.667$, $p < 0.05$]. When baseline body weight was used as a covariate, obese [$F(1, 55) = 41.908$, $p < 0.05$] and male [$F(1, 55) = 76.461$, $p < 0.05$] rats remained heavier than lean and female rats during the Post-stress Phase. Lean males weighed more than did lean females [$F(1, 27) = 19.815$, $p < 0.05$]. Obese male rats weighed more than did obese female rats [$F(1, 27) = 28.575$, $p < 0.05$]. There were no differences in body weight between the stress and control groups within each body type.

Repeated-measures analyses. All repeated-measures analyses indicated that during the Stress and Post-stress Phases, obese rats always weighed more than lean rats and males always weighed more than females. There were no interactions of Stress with Time or Sex, indicating that any effect of stress on body weight was not detectable statistically.

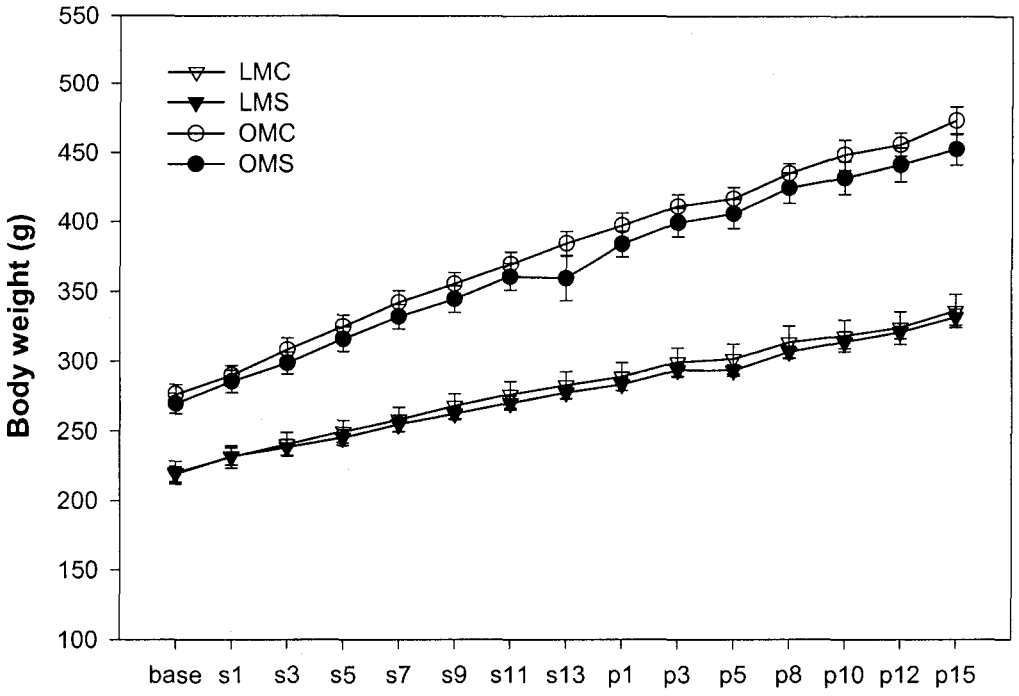

Figure 20. Body weight for males.

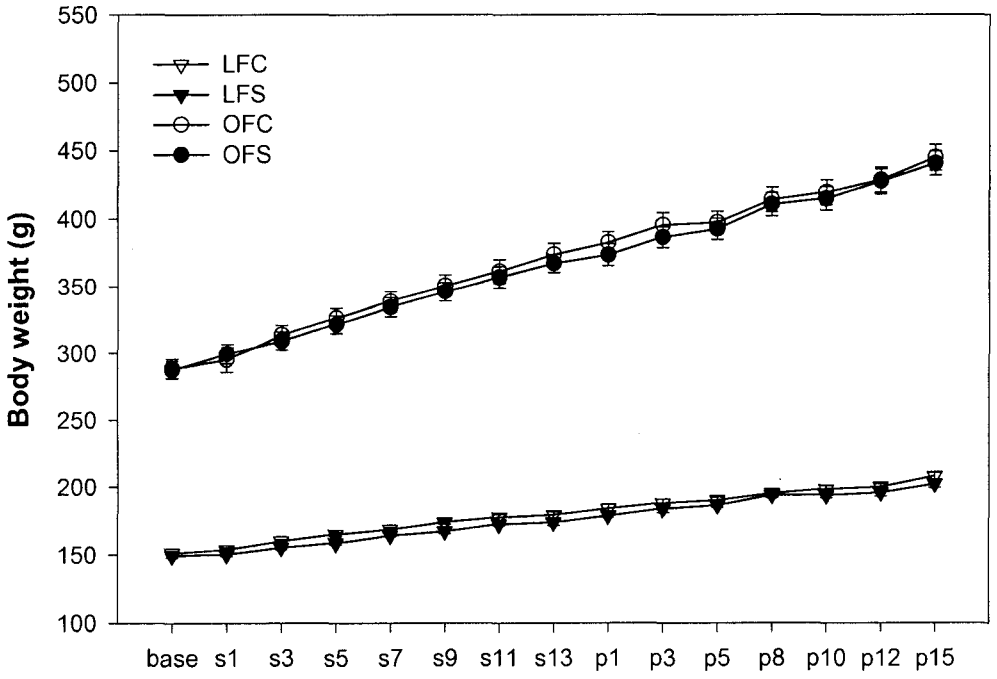

Figure 21. Body weight for females.

Lee Index

Obese had more body fat than lean rats [$F (1, 55) = 907.135$, $p < 0.05$] and males had more body fat than did females [$F (1, 55) = 90.994$, $p < 0.05$]. The Sex X Body Type interaction revealed that obese males had more body fat than did all other groups [$F (1, 55) = 44.778$, $p < 0.05$]. There were no differences in the Lee Index between the stress and control groups within each body type.

Corticosterone

Figure 22 shows the corticosterone data. It is important to note that animals in the stress groups were not re-exposed to the stressor prior to sacrifice; their last immobilization was 14 days prior to sacrifice. Overall, females had higher levels of corticosterone at the time of sacrifice than did males [$F (1, 54) = 4.301$, $p < 0.05$]. Overall, obese rats had higher levels of corticosterone than did lean rats [$F (1, 54) = 4.601$, $p < 0.05$]. Interestingly, rats without prior stress exposure had higher levels of corticosterone than did rats with prior stress exposure [$F (1, 54) = 9.192$, $p < 0.05$]. Obese unstressed rats had higher corticosterone levels than did obese stressed rats [$F (1, 27) = 6.943$, $p < 0.05$]. When examined separately, there were no differences in corticosterone levels between the stress and control groups within the lean rats. Lean females had greater corticosterone than did lean males [$F (1, 27) = 7.448$, $p < 0.05$].

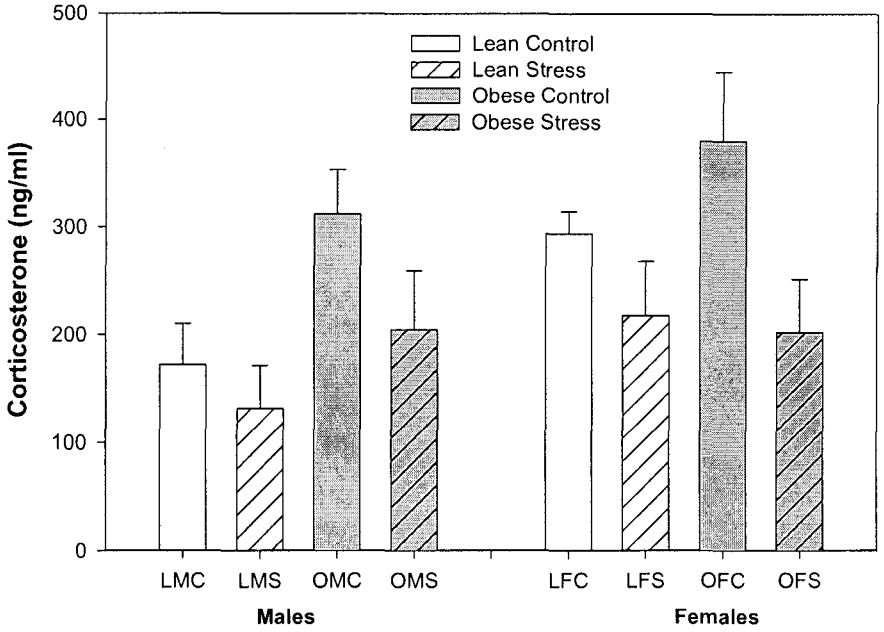

Figure 22. Corticosterone levels at time of sacrifice.

Summary of Biological Data

Obese and males weighed more than lean and female rats, respectively, during the Stress and Post-stress Phases. There were no differences in body weight between stressed and unstressed obese rats or lean rats across experimental phases, indicating the impact of repeated, acute stress on body weight was minimal. These findings replicate findings of Experiment I that indicated that stress did not alter body weight in obese Zucker males fed bland chow only.

Corticosterone levels were measured 14 days after the stressor terminated. Corticosterone data, therefore, reflect history of stress exposure rather than acute responses to the stressor. Obese rats and female rats had higher corticosterone levels than did lean rats and male rats. Interestingly, obese rats of both sexes with prior exposure to restraint stress had lower corticosterone levels than did rats

without prior stress exposure. The pattern was similar for lean animals of both sexes, but not statistically significant. These findings are in contrast to Experiment I findings in which lean animals had higher corticosterone levels than obese animals. However, lean animals from Experiment I were of the Sprague-Dawley strain – not the Zucker strain used in the present experiments. Findings for history of stress exposure are new; this was not assessed in Experiment I.

BEHAVIORAL DEPENDENT VARIABLES

The appendix provides descriptives and statistical analyses for all behavioral dependent variables. Figures 23 and 24 show the amount of food consumed at all phases.

Food consumption

Baseline Phase. Obese rats ate more food than lean rats [$F(1, 56) = 461.478$, $p < 0.05$]. Males consumed more food than females [$F(1, 56) = 26.232$, $p < 0.05$]. The Sex X Body Type interaction revealed that obese males and females consumed more food than did all other groups at baseline [$F(1, 56) = 10.739$, $p < 0.05$]. There were no differences in food consumption between rats assigned to the stress and control groups. There were no differences in food consumption between the stress and control groups within lean or obese rats at baseline.

Stress Phase. Obese rats ate more food compared to lean rats [$F(1, 56) = 386.719$, $p < 0.05$]. Male rats ate more food than did female rats [$F(1, 56) = 55.079$, $p < 0.05$]. The Stress X Sex interaction revealed that stressed males ate less than the other groups [$F(1, 56) = 5.530$, $p < 0.05$]. When baseline food consumption was used as a covariate, obese rats ate more food compared to lean rats [$F(1, 55) = 14.237$, $p < 0.05$]. Male rats ate more food than did female rats [$F(1, 55) = 22.379$, $p < 0.05$]. Stressed rats consumed less food than did unstressed rats [$F(1, 55) = 4.142$, $p < 0.05$]. There was no difference in food consumption between the stress and control groups within lean or obese rats during the Stress Phase.

Post-stress Phase. Obese rats ate more food compared to lean rats [$F(1, 56) = 456.500$, $p < 0.05$]. Male rats ate more food than did female rats [$F(1, 56) = 72.997$ $p < 0.05$]. The Body Type X Sex interaction indicated that obese male rats ate more than did all other rats [$F(1, 56) = 5.305$, $p < 0.05$]. When baseline food consumption was used as a covariate, obese rats ate more food compared to lean rats [$F(1, 55) = 24.031$, $p < 0.05$]. Male rats ate more food than did female rats [$F(1, 55) = 35.122$, $p < 0.05$]. There was no difference in food consumption between the stress and control groups within lean or obese rats during the Post-stress Phase.

Repeated-measures analyses of food consumption during Stress Phase.

Repeated-measures analyses indicated that during the Stress and Post-stress Phases, obese rats always ate more than lean rats and males always ate more than females. There were no interactions of Stress with Time, Body Type, or Sex, indicating that any effect of stress on feeding was minimal. It is worth noting, however, that food consumption overall decreased during the Stress Phase [$F(5, 275) = 3.770$, $p < 0.05$] and increased during the Post-stress Phase [$F(5, 270) = 2.875$, $p < 0.05$].

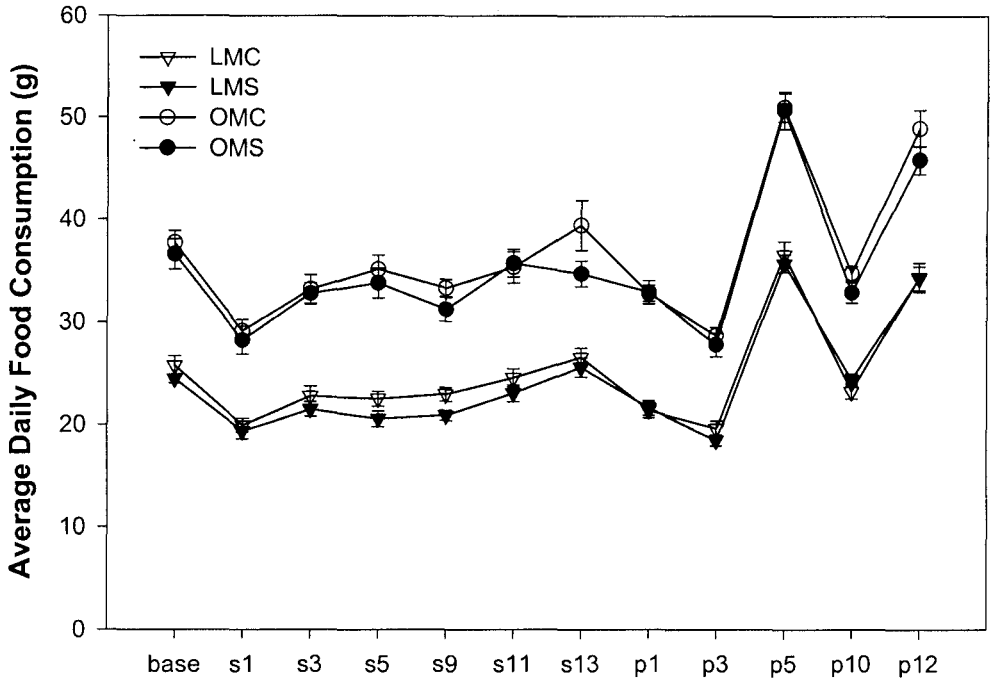

Figure 23. Average daily food consumption for males during all phases.

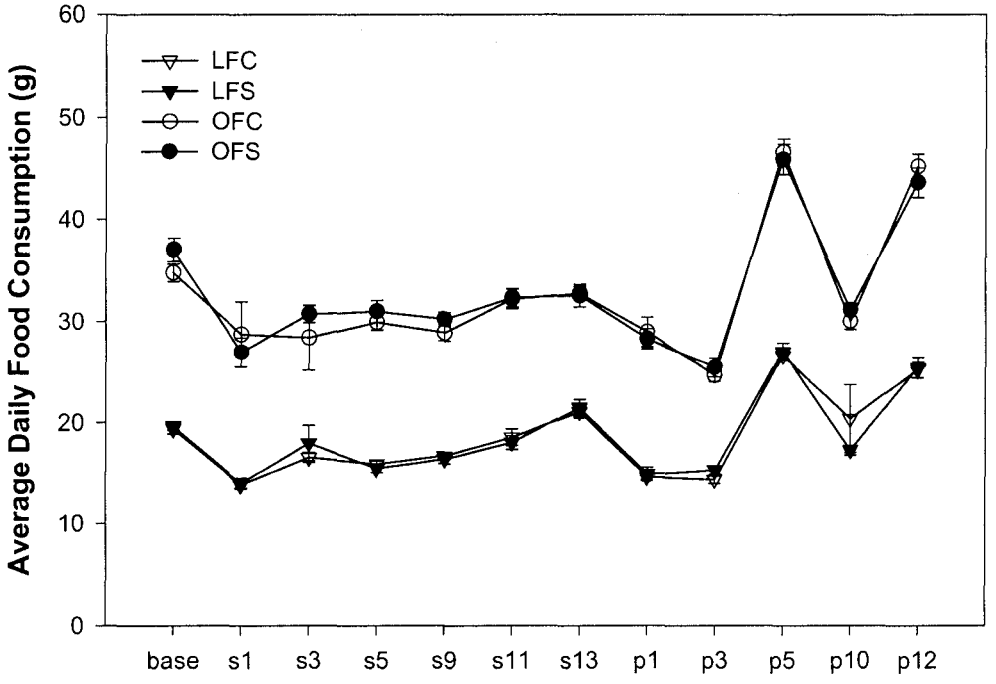

Figure 24. Average daily food consumption for females during all phases.

Food consumption summary. Food consumption decreased during the Stress Phase and increased during the Post-stress Phase. Obese and male rats consumed more food than did lean and female rats during all phases of the experiment.

Home Cage Activity (HCA)

Figures 25 and 26 show the levels of home cage activity during each phase.

Baseline Level of HCA. Obese rats had lower levels of activity than did lean rats [$F(1, 56) = 134.823$, $p < 0.05$]. Females had greater levels of home cage activity than did males [$F(1, 56) = 14.526$, $p < 0.05$]. The Body Type X Sex interaction revealed that lean females had greater levels of home cage activity than all other groups [$F(1, 56) = 5.727$, $p < 0.05$]. When each body type was examined separately, there were no differences in level of home cage activity at baseline between rats assigned to the stress or control groups.

Stress Phase. Level of HCA. Lean rats had greater levels of HCA than did obese rats during the Stress Phase [$F(1, 55) = 10.621$, $p < 0.05$]. Stressed rats had greater home cage activity levels than did unstressed rats [$F(1, 55) = 18.772$, $p < 0.05$]. The Sex X Stress interaction revealed that stressed females had the highest and unstressed females the lowest levels of HCA compared to other groups [$F(1, 55) = 11.579$, $p < 0.05$]. The Body Type X Sex interaction indicated that lean females had the greatest level of HCA compared to all others [$F(1, 55) = 10.248$, $p < 0.05$].

Among lean rats, females had greater levels of HCA than did males during the Stress Phase [$F(1, 27) = 4.634$, $p < 0.05$]. Lean stressed rats had greater

levels of HCA than did unstressed lean rats [$F(1, 27) = 5.967$, $p < 0.05$]. Lean stressed females had somewhat greater levels of HCA than did all other lean rats [$F(1, 27) = 3.379$, $p = 0.077$]. Among obese rats, males had somewhat greater levels of HCA than did females [$F(1, 27) = 3.883$, $p = 0.059$]. Obese stressed rats had greater levels of HCA than did obese unstressed rats [$F(1, 27) = 14.327$, $p < 0.05$]. The Sex X Stress interaction revealed that obese stressed females had greater HCA than did all other obese rats [$F(1, 27) = 9.088$, $p < 0.05$].

Post-stress Phase. Level of HCA. Lean [$F(1, 55) = 12.827$] and stressed [$F(1, 55) = 21.199$, $p < 0.05$] rats had greater levels of HCA than did obese and unstressed rats, respectively, during the Post-stress Phase. The Body Type X Stress interaction revealed that lean stressed rats had greater levels of HCA than lean unstressed and obese stressed and unstressed rats [$F(1, 55) = 12.800$, $p < 0.05$]. These findings are best explained by the Body Type X Sex X Stress interaction such that lean stressed males had greater levels of HCA than did all other groups [$F(1, 55) = 4.494$, $p < 0.05$].

Among lean rats, stressed rats had greater HCA than did unstressed rats during the Post-stress Phase [$F(1, 27) = 31.636$, $p < 0.05$]. The Sex X Stress interaction revealed that lean stressed males had greater HCA than did all other lean rats [$F(1, 27) = 7.728$, $p < 0.05$]. Among obese rats, there were no differences between the stress and control groups.

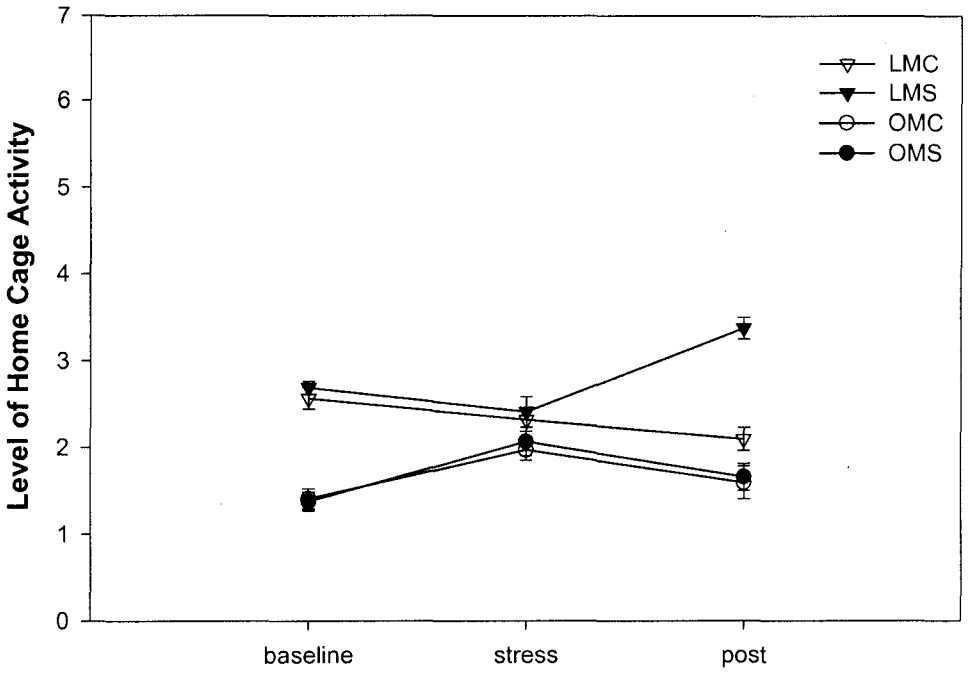

Figure 25. Home cage activity in males during all phases.

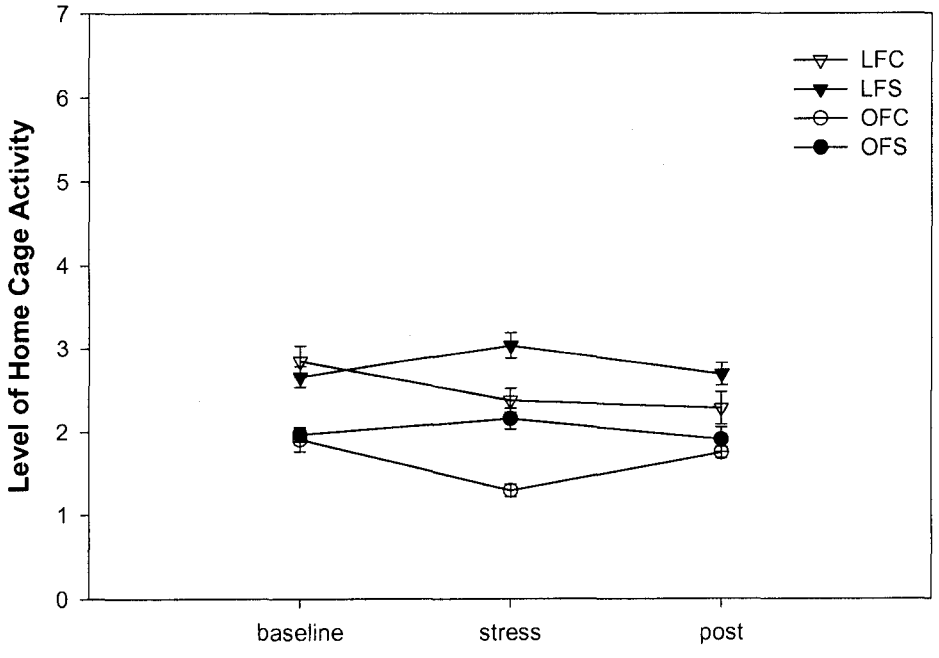

Figure 26. Home cage activity in females during all phases.

Repeated-measures analyses of HCA Level during Stress and Post-stress Phases. Lean [$F(1, 55) = 17.956$, $p < 0.05$] and stressed [$F(1, 55) = 30.624$, $p < 0.05$] rats had greater levels of HCA than did obese and unstressed rats, respectively. The Time X Body Type X Sex interaction revealed that lean females had greater levels of HCA during Stress Phase and that lean males had greater levels of HCA during Post-stress Phase than all other groups [$F(1, 55) = 17.059$, $p < 0.05$]. The Time X Body Type X Stress interaction indicated that lean stressed rats had greater levels of HCA during Stress Phase and Post-stress Phase than all other groups [$F(1, 55) = 12.489$, $p < 0.05$]. The Time X Sex X Stress interaction revealed that stressed females had greater HCA during Stress Phase and stressed males had greater HCA during Post-stress Phase compared to all groups [$F(1, 55) = 18.966$, $p < 0.05$].

Baseline Phase. Home cage behaviors. During the Baseline Phase, fewer obese rats engaged in vertical activity [$\chi^2(1) = 20.390$, $p < 0.05$] and obese rats slept more than lean rats [$\chi^2 = 11.201$, $p < 0.05$]. More rats assigned to the control group slept than rats assigned to the stressed group during baseline [$\chi^2(1) = 7.654$, $p < 0.05$]. There were no differences in specific home case behaviors between males and females at baseline.

Stress and Post-stress Phase. Home cage behaviors. During both phases, obese rats were more likely to be eating, sedentary (awake not moving), and sleeping than lean rats. Lean rats were more likely to be engaged in grooming, horizontal activity, and vertical activity than were obese rats. Females generally were more active and slept less than males. During the Post-stress Phase, animals

with prior stress exposure were more likely to be engaged in grooming behaviors than control rats

Home cage summary. Obese rats had lower levels of home cage activity and engaged in more sedentary behaviors than did lean rats at all phases. Lean and obese stressed females had greater levels of home cage activity than did their same-body type stressed males during the Stress Phase. Lean stressed males had greater home cage activity during the Post-stress Phase.

Locomotor Open Field (OF)

Figures 27-29 show the locomotor activity.

Baseline Phase (OF). Obese rats had less horizontal activity [$F(1, 56) = 46.491$, $p < 0.05$] and vertical activity [$F(1, 56) = 35.242$, $p < 0.05$] than did lean rats. The Body Type X Stress interaction indicated that lean rats assigned to the unstressed group had greater vertical activity [$F(1, 56 = 5.616$, $p < 0.05$] than all other groups. Obese female rats had greater vertical activity than obese male rats [$F(1, 28) = 4.066$, $p < 0.05$]. Obese females assigned to the control group had greater vertical activity than did all other obese rats [$F(1, 28) = 4.066$, $p < 0.05$].

Stress and Post-stress Phases (OF). Given the many baseline differences in activity level, all subsequent analyses were run with baseline values as covariates. Overall, obese animals, regardless of sex, exhibited extremely low levels of horizontal and vertical activity across experimental phases. In addition, there were no changes in horizontal and vertical activity from the Stress Phase to the Post-stress Phase among obese male and female rats. In contrast, lean male and female animals increased horizontal and vertical activity during each phase.

Among males, lean stressed males had higher horizontal activity levels than did lean unstressed males during the Post-stress Phase.

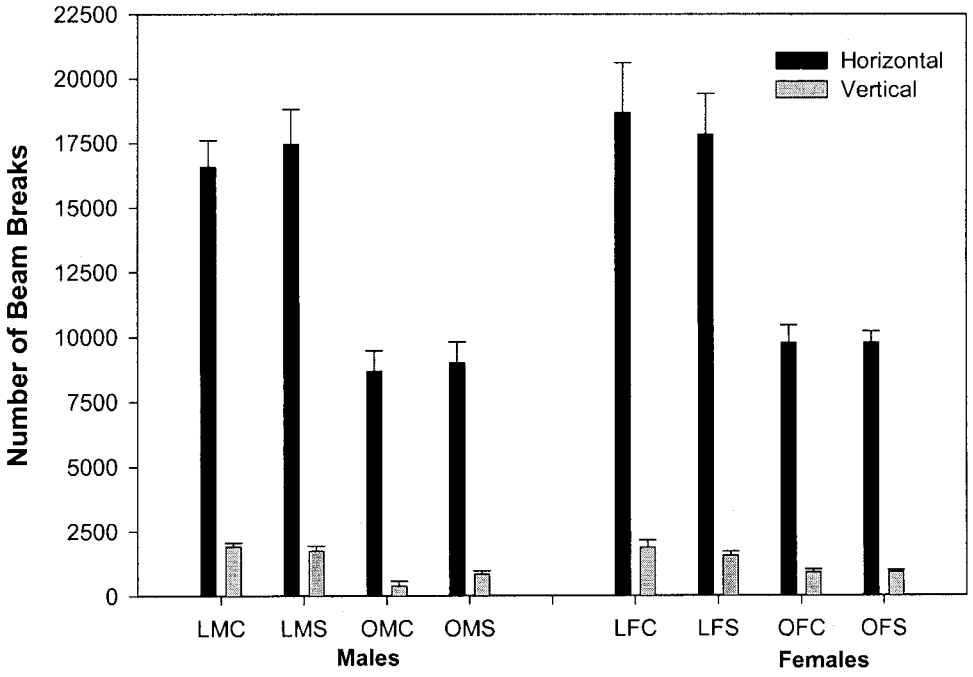

Figure 27. Horizontal activity and vertical activity during Stress Phase.

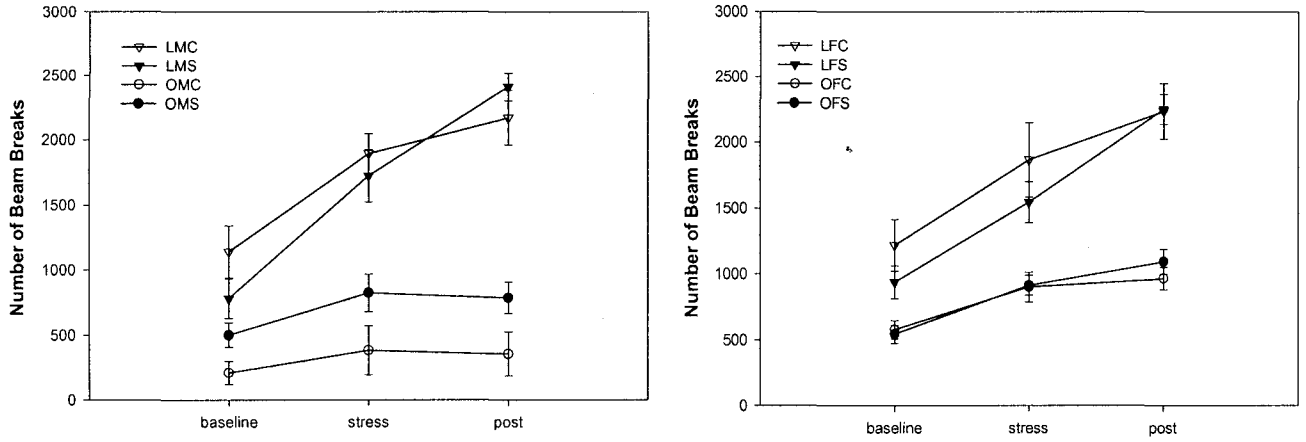

Figure 28. Vertical activity during all phases for males (left) and females (right).

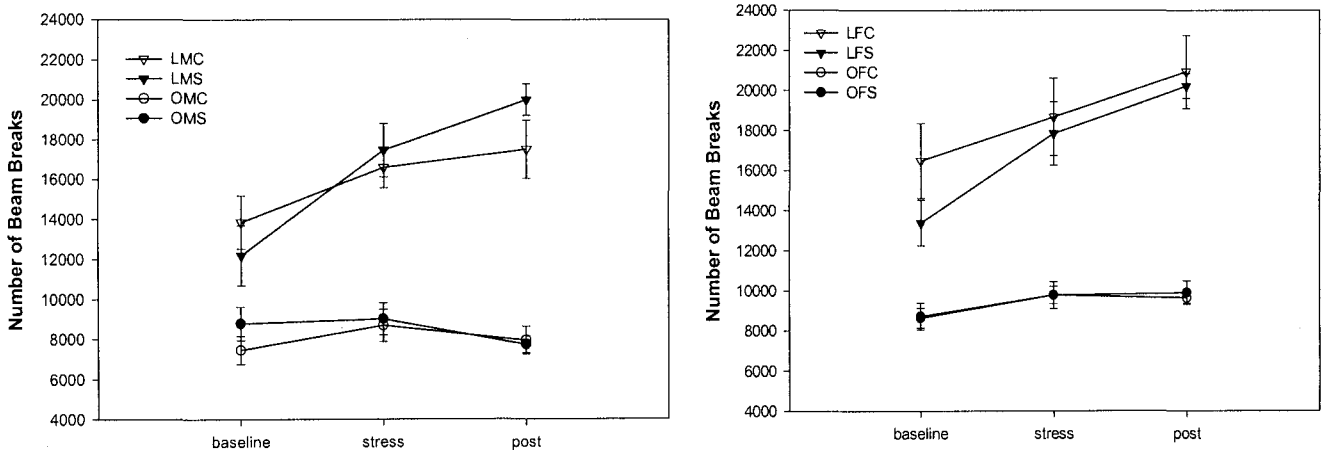

Figure 29. Horizontal activity during all phases for males (left) and females (right).

Locomotor (OF) summary. Obese male and female rats had less horizontal and vertical activity at all phases of the experiment compared to lean rats and, importantly, their activity levels did not change in response to stress or in the Post-stress Phase. Lean animals had higher activity levels throughout the experiment and lean stressed males in particular exhibited increased activity in the Post-stress Phase.

COGNITIVE DEPENDENT VARIABLES

The appendix provides descriptives and statistical analyses for the cognitive dependent variables. Figures 30 – 36 show the startle responses and percent pre-pulse inhibition.

Acoustic Startle Response (ASR) with and without Pre-pulse Inhibition (PPI)

Startle Responses. (See appendix for details of analysis). At baseline, lean males assigned to the stress group had somewhat higher startle responses to the 110 and 120 dB stimuli. Therefore, all subsequent analyses were run with baseline values as covariates. In addition, at baseline lean males had higher startle responses than obese males across stimuli. Among females, however, the pattern is reversed with obese females exhibiting greater startle responses to the 120 dB stimulus than lean females.

From the baseline to the Stress Phase, animals generally maintained the same amplitude of startle or increased slightly. Among males, lean animals continued to startle more than obese animals, regardless of stress condition. Among females, the reverse pattern also held, with obese females startling more than lean females. In addition, lean control females startled more than lean stressed females.

From the Stress Phase to the Post-stress Phase, generally animals increased startle amplitudes. Among males, lean rats continued to startle more than obese rats. In addition, a post-stress effect appeared in which lean stressed males startled more than lean unstressed males to both stimuli. Among obese males, there were no differences based on prior stress exposure. Among females, obese females

continued to startle more than lean females with obese unstressed females startling more than obese stressed females. Among lean females, there were no differences based on prior stress exposure.

Percent PPI (%PPI) Responses. Patterns were different for males compared to females. For males, generally %PPI responses increased over phases. There were however, no differences based on body type or stress exposure during the Stress Phase. In the Post-stress Phase, however, lean males (regardless of stress exposure) exhibited greater %PPI than obese males.

Among females, there were large differences in baseline %PPI responses, with obese control females exhibiting the highest %PPI levels; the difference was of such magnitude that interpreting this group relative to the other groups, even with covaried baseline values, is difficult. There were no clear differences among groups during the Stress Phase. During the Post-stress Phase, among lean females, the stressed group had greater %PPI responses than the unstressed group. Lean stressed females had increased %PPI and obese stressed females had decreased %PPI [$F(1, 27) = 3.962$, $p = 0.057$].

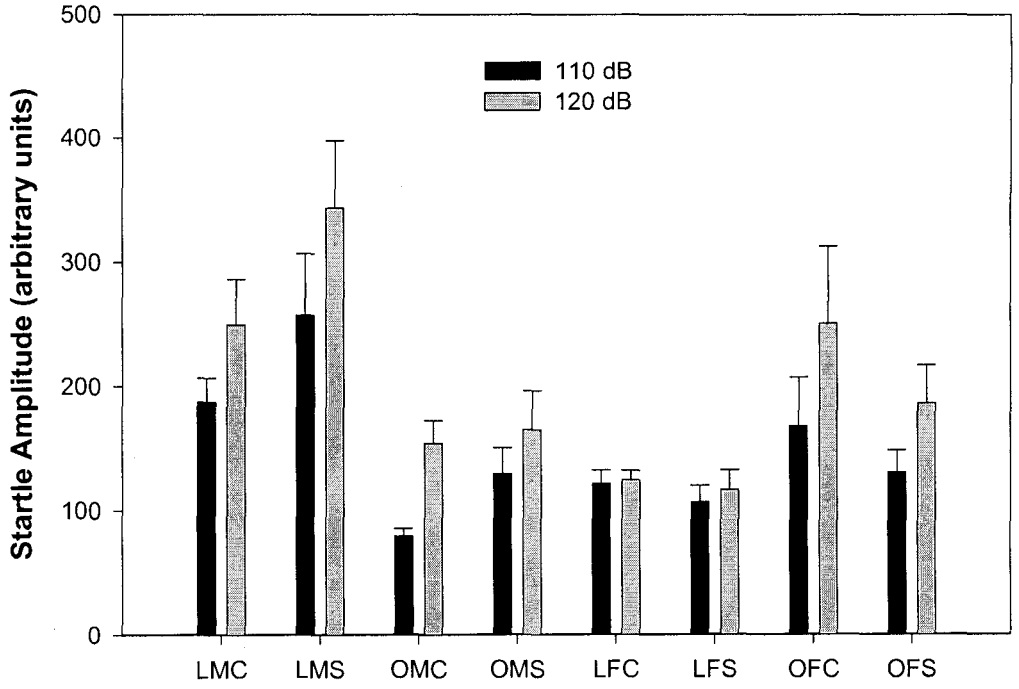

Figure 30. Startle amplitude to 110 dB and 120 dB stimuli during Baseline Phase.

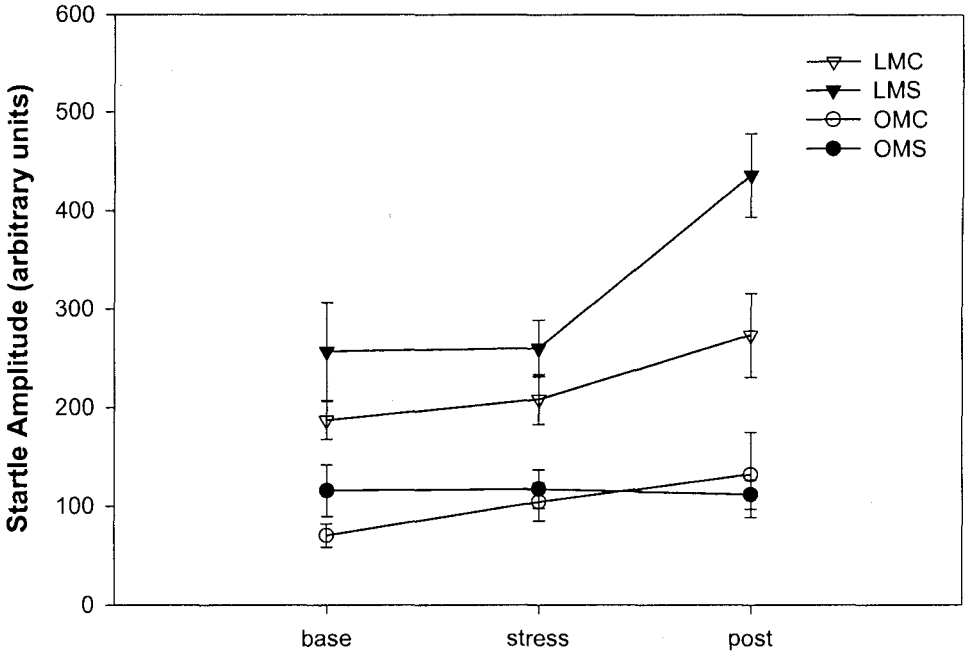

Figure 31. Startle amplitude to 110 dB for males.

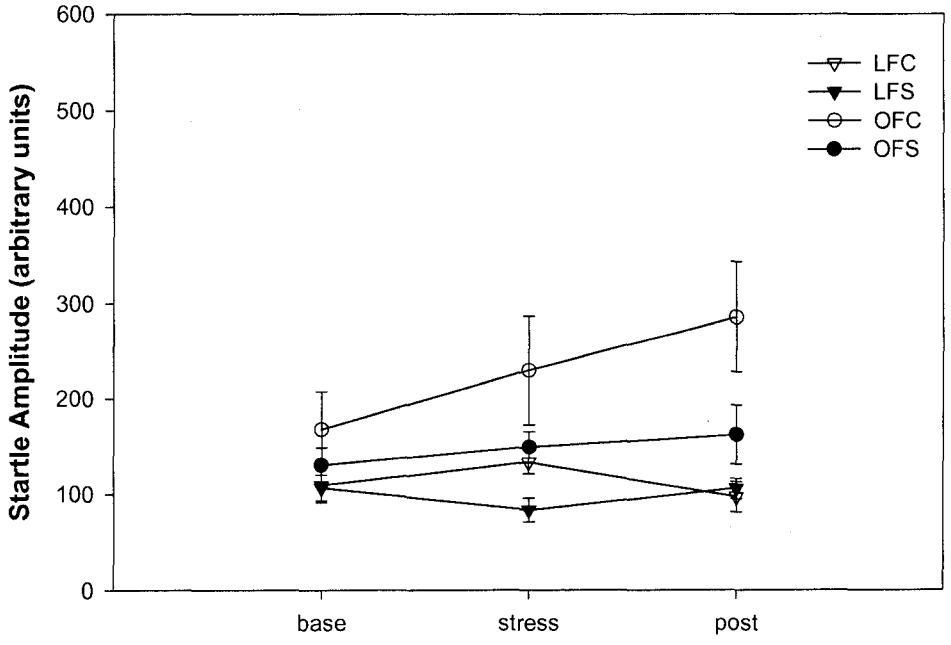

Figure 32. Startle amplitude to 110 dB for females.

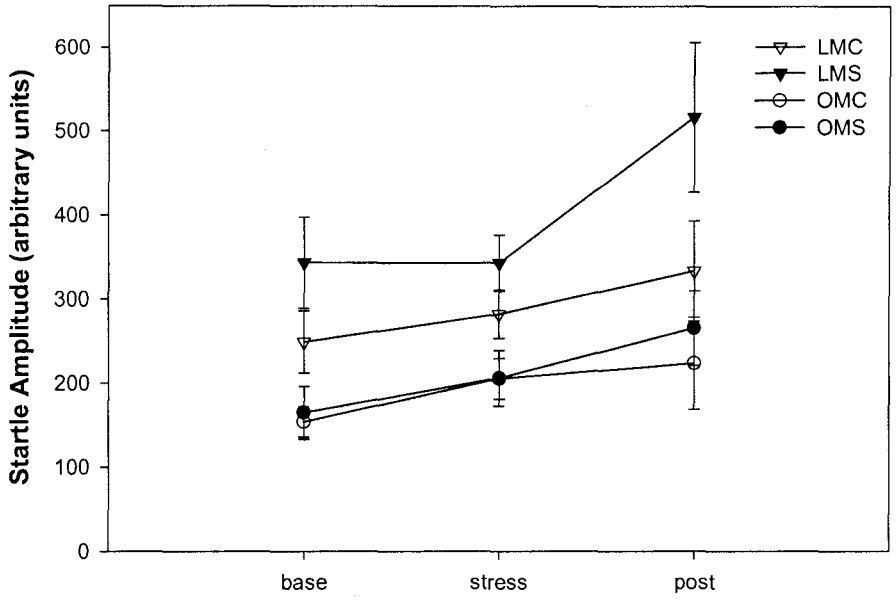

Figure 33. Startle amplitude to 120 dB stimulus for males.

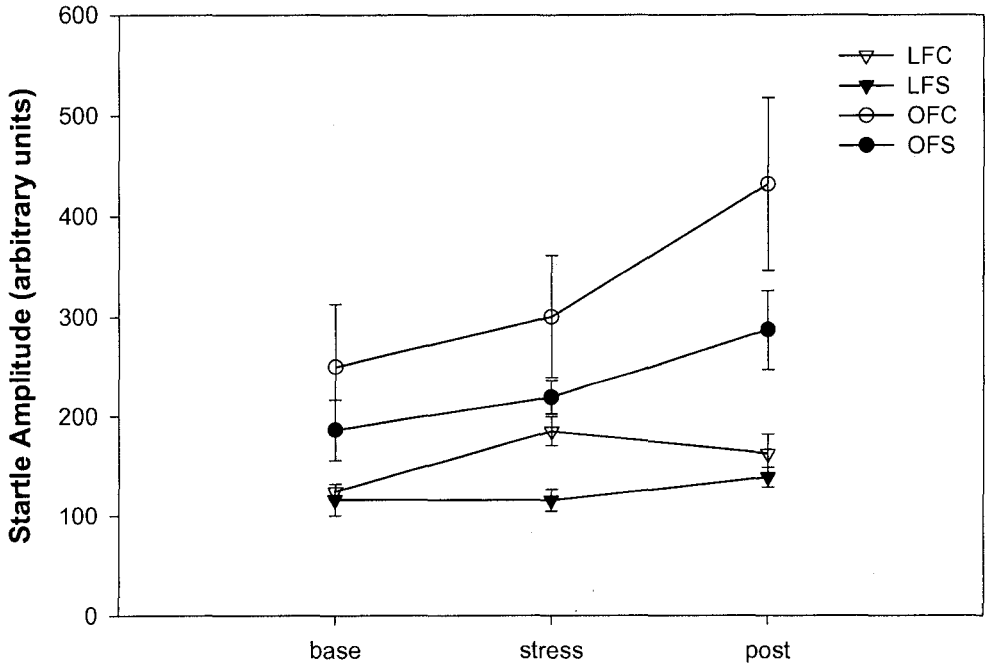

Figure 34. Startle amplitude to 120 dB stimulus for females.

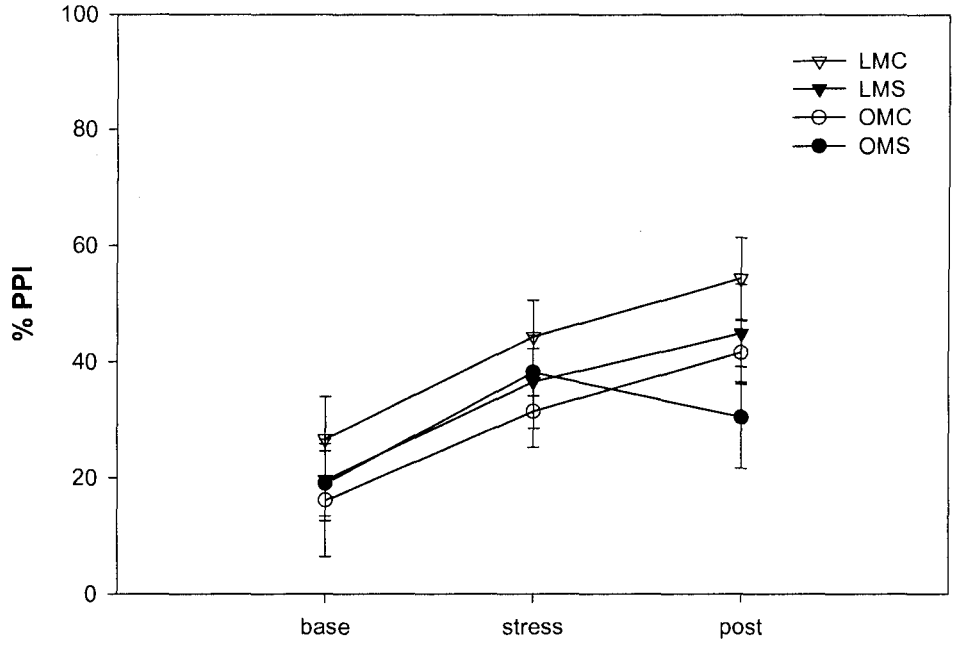

Figure 35. Average % PPI for males.

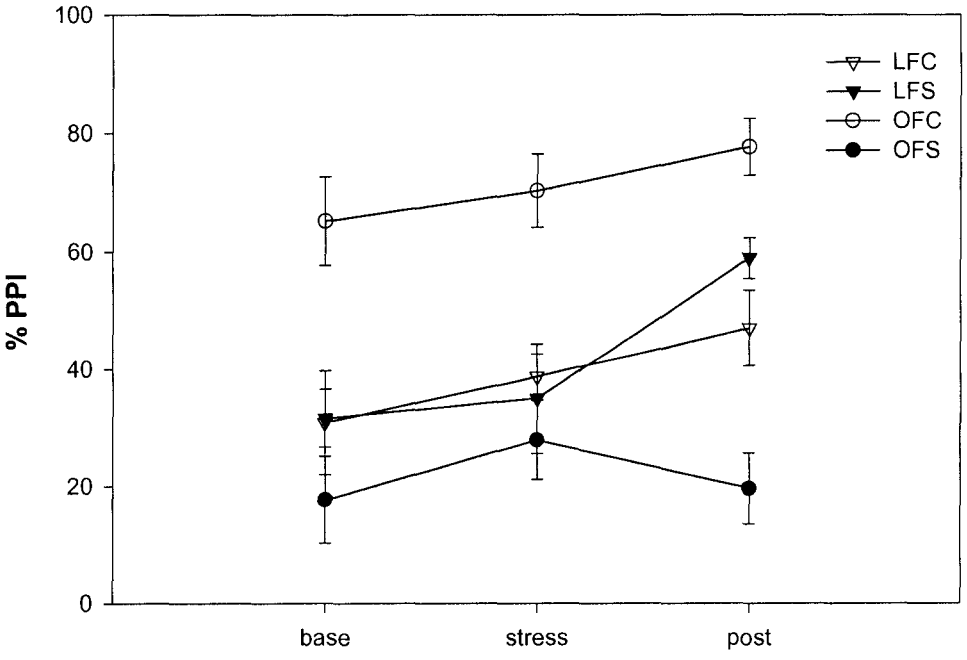

Figure 36. Average %PPI for females.

ASR and PPI Summary. With regard to ASR, there were body type and sex differences in responses. Lean males startled more than obese males across all experimental phases, with lean stressed males startling more than lean unstressed males in the Post-stress Phase. For females, obese rats startled more than lean rats across all phases. During the Post-stress Phase, obese control females startled more than did obese stressed females.

With regard to PPI responses, in the Post-stress Phase, lean males (regardless of stress exposure) exhibited greater %PPI than obese males. Among females, lean stressed females exhibited greater %PPI responses than lean control females and much greater %PPI responses than obese stressed females.

ASSESSMENT OF EXPERIMENT II HYPOTHESES

Hypothesis 1a. Obese rats and lean rats will decrease food consumption in response to stress but will continue to gain weight consistent with growth curves for each strain.

Supported. Overall, obese rats were heavier than lean rats throughout the experiment and exhibited greater rates of body weight gain. Stress decreased food consumption in both strains but did not alter rates of body weight gain.

Hypothesis 1b. Obese rats will have a blunted corticosterone response compared to lean rats.

Not Supported. Obese rats and female rats had higher corticosterone levels than did lean rats and male rats. Obese rats of both sexes with prior exposure to restraint stress had lower corticosterone levels than did rats without prior stress exposure. The pattern was similar for lean rats of both sexes, but not statistically significant.

Hypothesis 1c. Obese rats will have lower levels of physical activity overall compared to lean rats and will not alter physical activity responses when exposed to stress (e.g., horizontal and vertical activity, and home cage activity); lean rats will increase activity levels in response to stress.

Supported. Obese rats had lower levels of horizontal and vertical activity and lower levels of home cage activity compared to lean animals and these activity patterns were not affected by stress. Lean rats increased activity levels in response to stress during the Post-stress Phase.

Hypothesis 1d. Stress will increase startle responses in lean rats compared to obese rats without altering percent PPI.

Partially Supported. Among lean males, prior exposure to stress increased startle responses to the 110 and 120 dB stimuli in the Post-stress Phase. Patterns among females were different. Among females, obese stressed females had increased startle responses to 120 dB and lean stressed females had unchanged startle responses in the Post-stress Phase. Lean stressed females increased %PPI levels and obese stressed females decreased %PPI levels in the Post-stress Phase.

Specific Aim #2: *Determine the effect of sex on biobehavioral responses to repeated acute stress in obese and non-obese rats*

Hypothesis 2. Stress will have different effects on behavioral and biological responses of male and female, obese and non-obese rats.

Partially Supported. There were no clear gender differences in body weight and food consumption responses based on body type or stress exposure. In the Post-stress Phase, the lean stressed males and obese unstressed females exhibited greater startle amplitudes. Lean males exhibited greater %PPI than did obese males during the Post-stress Phase. Lean stressed females exhibited greater %PPI than lean unstressed females and much greater %PPI than obese stressed females in the Post-stress Phase. Females also exhibited higher corticosterone levels than did males, across body types, and independent of stress.

DISCUSSION: EXPERIMENT II

The purpose of Experiment II was to determine whether behavioral and biological responses to stress differ based on body type and sex. Lean and obese rats of the same strain were used. Males and females representing both body types were randomly assigned to either a control or stress condition. Rats assigned to the stress condition were restrained for 20 minutes on 14 consecutive days. Responses also were measured during a Post-stress Phase.

Effects of Stress. The major effects of stress occurred during the Post-stress Phase. These included: (1) prior stress exposure resulted in lower corticosterone levels compared to a no-stress history and these effects were similar for both lean and obese Zucker rats; (2) startle amplitude among lean males was increased by prior stress exposure whereas the greatest startle responses among females occurred for obese unstressed animals; (3) lean stressed females exhibited greater %PPI than lean unstressed females and than obese stressed females. These findings illustrate the importance of measuring responses during a Post-stress Phase.

The most striking finding overall, however, is the relative lack of response to a repeated, acute stressor. There were no changes in body weight or food consumption among either body type and no activity changes (horizontal and vertical) among obese rats that could clearly be attributed to the stress manipulation. Overall, the Zucker strain, and particularly the obese Zuckers, appeared relatively stress-insensitive behaviorally.

Another noteworthy finding is corticosterone differed based on body type and prior stress exposure. Obese unstressed rats had greater levels of corticosterone than lean unstressed rats which is consistent with the literature that obesity is associated with elevated corticosterone levels. It was an unexpected finding that 14 days after restraint ended male and female obese rats with prior exposure to restraint stress had lower corticosterone levels than did male and female obese rats without prior stress exposure. The same pattern was observed among lean rats but the difference was not significant. Other investigators have reported similar findings such that 5 days after repeated acute restraint ended lean male rats (Wistar strain) with a stress history had lower corticosterone levels compared to controls (Harris et al., 2002). The implication is that repeated stress results in lasting changes in HPA axis hormone levels and that these changes may last even longer in obese individuals. Note that the corticosterone data in Experiment II are comparable to those reported in another study of effects of stress in lean and obese, male and female Zuckers conducted by Guillame-Gentil and colleagues (1990).

Sex Differences. It is difficult to clearly delineate consistent sex differences across measures and in response to stress. In many instances, the impact of body type appeared to be greater than the effect of sex. However, responses to stress among males and females differed during the Post-stress Phase on some measures. Lean males startled more than obese males throughout the experiment; obese females, however, startled more than did lean females. In the Post-stress Phase, the lean stressed males and obese unstressed females exhibited greater startle amplitudes. Lean males exhibited greater percent PPI than did obese males

during the Post-stress Phase. Lean stressed females exhibited greater percent PPI than lean unstressed females and much greater percent PPI than obese stressed females in the Post-stress Phase. Females also exhibited higher corticosterone levels than did males, across body types. Overall, the Sex X Stress interactions appeared on only a few measures and only in the Post-stress Phase.

Overall, Experiment II indicated that the Zucker strain is relatively insensitive to behavioral effects of mild repeated stress, that body type overshadows most differences based on sex, and that biological responses may reveal lasting stress effects that are not evident behaviorally.

SECTION III: ASSESSMENT AND GENERAL DISCUSSION

Two separate experiments were included in this doctoral dissertation research project to examine the behavioral and biological effects of repeated stress as a function of body weight, diet, and sex. Experiment I examined behavioral and biological effects of repeated acute stress on obese Zucker and non-obese Sprague-Dawley male rats. The findings from Experiment I established that the genetic-based model produced rats that were significantly heavier in a shorter time period than did a diet-induced model of rodent obesity. Experiment II expanded on Experiment I by using the genetic-based model of obesity to examine male and female, obese and non-obese Zucker rats in stressed and unstressed conditions.

The major effects of stress in Experiment I were: (1) decreased bland food consumption in both strains; (2) altered cafeteria food consumption with increased chip consumption among Zuckers and decreased cookie consumption among Sprague-Dawleys; (3) reduced overall kilocalorie consumption in all groups except for Zuckers fed the cafeteria diet; (4) greater corticosterone levels among lean rats re-exposed to stress; (5) greater levels of physical activity in familiar and novel environments among lean rats; and (6) greater startle responses among lean rats but impaired attentional processing among animals fed the cafeteria diet.

The only effect of stress in Experiment II that was evident during the Stress Phase was increased activity levels among lean Zucker rats. The major effects of stress in Experiment II occurred during the Post-stress Phase. These included: (1) prior stress exposure resulted in lower corticosterone levels compared to a no-stress history and these effects were similar for both lean and obese Zucker rats; (2) startle

amplitude among lean males was increased by prior stress exposure, whereas the greatest startle responses among females occurred for obese unstressed rats; (3) lean stressed females exhibited greater %PPI than lean unstressed females and than obese stressed females. These findings illustrate the importance of measuring responses during a Post-stress Phase. The implication is that male and female, lean and obese rats also differ in the amount of time necessary to recover from stress.

Some findings of Experiments I and Experiment II were similar: (1) body weight was not affected by stress; (2) stress decreased bland food consumption; (3) obese rats did not alter physical activity in response to stress. These findings are not consistent with the literature that found stress in obese rats is associated with greater body weight gain without a change in food consumption (Levin et., 2000; Michel, Levin, & Dunn-Meynell, 2003) and greater levels of physical activity (i.e., horizontal activity) compared to lean stressed rats (Michel, Levin, & Dunn-Meynell, 2003). The discrepancies may be partially attributed to differences in stress manipulation, diet, rat strain, and length of time physical activity measured.

The finding that stressed animals decreased bland food consumption but did not lose weight warrants further discussion. First, it should be noted that animals in both experiments were in a dynamic growth phase because they were in young adulthood. All animals were 20-30 days old when they arrived and were about 11-12 weeks old when the experiment concluded. The breeders expect animals to grow until about 15 weeks old. Second, restraint stress is associated with modest reductions in body weight of 5-10% (Harris et al., 2002). Taken together, it is not

surprising that the modest effects of stress on body weight did not circumvent the dynamic growth phase. It is noteworthy that stress-induced reductions in body weight and food consumption generally are restored once the stressor ends in animals that are not in a dynamic growth phase (Harris et al., 2002). Also, differences in baseline body weights and food consumption in Experiments I and II are attributable to slight differences in age at the beginning of each experiment.

Some findings in the two experiments were different. In Experiment I, lean rats had greater startle responses than obese rats in response to stress. In Experiment II, when background genetic strain was controlled by using only Zuckers, startle responses did not differ between lean and obese rats during the Stress Phase but during the Post-stress Phase lean stressed males had greater startle amplitudes compared to lean unstressed males. Percent pre-pulse inhibition differed based on sex and body type during the Post-stress Phase. Obese females may be more sensitive to the negative effects of stress on attentional processes based on a decrease in %PPI during the Post-stress Phase.

Implications for Individual Differences in Stress Reactivity Based on Body Type

The findings in the present research highlight two major important themes relevant to understanding potential causal factors in the stress and energy imbalance relationship. First, the findings indicate that the conceptual model in Figure 1 is useful to understand the role of body type in stress reactivity. In particular, obese animals were relatively behaviorally unresponsive to the stressful experience while it was ongoing. The failure to adapt to stress and modify behaviors

to compensate for the biological consequences of SNS and HPA activity can be conceptualized as not only maladaptive but also, ultimately, health-harming. Findings in Experiment I suggest that obese animals were biochemically sensitive to the restraint stress because corticosterone was elevated in response to the re-stress exposure. Experiment II findings add an important dimension to this finding. Although the obese animals were likely responsive to the restraint stressor during the Stress Phase in Experiment II, in the Post-stress Phase they exhibited marked reductions in corticosterone levels compared to obese animals without a history of stress exposure. These reductions, present 14 days after the stressor ceased, suggest that effects of mild stress in obese animals were long-lasting and produce abnormal metabolic and stress hormone rhythms long after the stressful experience ended. These findings suggest that stress recovery periods may be delayed among obese and that acute, repeated stress has lasting effects on biological responses that are critical in energy balance regulation. In addition, in humans, low corticosterone levels have been associated with psychiatric disorders such as post-traumatic stress disorder as well as with chronically-stressed states (Kanter et al., 2001).

Second, one of the most important findings from Experiment I is that stress clearly changed feeding behavior and food preferences in lean and obese rats. In the presence of high-fat, salty, and sweet food choices, obese stressed animals increased consumption of salty, high-fat foods (e.g., chips) and maintained consumption of sweet, high-fat foods (e.g., cookies). This pattern is in contrast to lean stressed animals; these animals decreased consumption of all food types in

response to stress. This finding indicates that the food options presented in the environment are a powerful determinant of what stressed, obese organisms consume.

The decrease in sweet- and salty-tasting foods among lean rats is consistent with the anhedonia hypothesis which suggests that stress decreases appetitive activity (i.e., "pleasurable foods") (Pecoraro et al., 1994). The present findings imply that the anhedonia hypothesis applies to lean rats only. However other studies have found that lean Sprague-Dawleys increased consumption of sweet-tasting foods (e.g., Pecoraro et al., 2004). Some investigators speculate that pre-existing dietary habits are important predictors of how energy will be regulated during stressful periods. The regulatory shift hypothesis indicates that animals that consume low-energy diets in normal, unstressed periods are likely to mobilize endogenous energy stores via lipolysis and fat oxidation during stressful periods (Dess, 1991; Dess, Choe, & Minor, 1998). The behavioral expression of this energy shift in animals typically maintained on a low-energy diet is decreased feeding and decreased body weight in response to stress. This hypothesis may partially explain why the lean animals in the present research reduced food intake of bland and cafeteria food. The feedforward hypothesis, which suggests that stress induces increased consumption of high caloric foods, may partially explain why chip consumption increased and cookie consumption remained about the same among obese rats.

The shift in preference toward salty-tasting foods further supports that obesity is associated with metabolic dysregulation. Some investigators have reported that elevated levels of aldosterone are common in obesity (Ehrhart-Bornstein et al.,

2003). Aldersterone, a mineralocorticoid produced by the adrenal cortex, signals the kidneys to conserve sodium and increase water retention. The increased chip consumption among obese rats may be indicative of disruption in the renin-angiotensin II-aldosterone system that was exacerbated by the onset of stress.

The two experiments suggest that obese organisms are more likely to be physically inactive and to consume health-harming foods in response to stress. As Cannon (1935) suggested, physical activity is a healthy coping behavior associated with fewer stress-related outcomes. If physical activity buffers stress, then obese individuals may have an increased vulnerability to develop stress-related outcomes because of a sedentary lifestyle and the propensity to consume health-harming foods.

One of the major focuses of Experiment II was to examine the potential role of sex differences in stress reactivity within and between body types. Overall, body type overshadowed any sex differences in response to stress, with the exception of startle and pre-pulse inhibition findings. These findings, which can be interpreted as Sex X Body type differences in cognitive responses, suggest that a more thorough assessment of sex differences in animals of different body types is needed. It may be that the primary sex differences in response to stress are in the attentional and information-processing domains. An experiment that focuses entirely on attention, short- and long-term memory, and other cognitive indices might more completely illuminate these differences.

FUTURE DIRECTIONS AND LIMITATIONS

Some of the experimental methods used may have limited the findings and should be considered when interpreting the results. First, in both experiments, the decision was made to use a mild, repeated stressor that also is non-painful. Subjects may have habituated to the stress manipulation, yet, restraint stress has been used for up to 21 days without behavioral or biochemical habituation in several strains to include Sprague-Dawleys (Faraday, 2002; Gamaro et al., 1998; Raygada et al., 1992). A more aversive stressful experience (i.e., tail shock, predator stress) or exposure to different stressors may have increased the magnitude of stress responses and more clearly revealed differences in stress reactivity between body types and between sexes within and across body types. It is unknown whether findings in these experiments would be replicated with more powerful stressors or if the stressor is varied and unpredictable. Future research should examine other stressors to address these questions.

One of the strengths of animal studies is that the environment can be controlled and manipulated in ways that are difficult or impossible when using humans as participants. Experiment I revealed that when obese animals are stressed and have access to unhealthy foods, consumption of high-fat salty foods increases and consumption of high-fat sweet foods is maintained while consumption of bland-tasting nutritionally-balanced food decreases. Because animals did not also have access to low-fat salty and sweet foods, it is not possible to separate the role of taste preference from macronutrient content. If animals also had access to

sweet fruit and salty low fat foods (such as pretzels), then consumption patterns of high-fat foods might have been different.

Some of the measurement procedures also may limit findings. For example, placing rats in the ASR cylinder may have evoked an additional stress response. The ASR cylinder is similar to the restraining device in that the rat cannot escape. Unlike the restraint device, the ASR cylinder allows the rat to make small movements. Movement in the ASR cylinders was restricted to a greater extent among the obese rats than among the lean rats. Lean rats could turn around in the ASR cylinder, whereas most obese rats could not. Therefore, placement in the ASR cylinder might have induced a stress response that was greater among obese rats than lean rats. However, because obese unstressed females had greater %PPI than all other animals, it is not likely that the logistics associated with ASR procedures affected obese rats differently than lean rats.

It also is possible that the instrument used to measure home cage activity lacked sensitivity to capture true differences in stress responses between lean and obese rats because of the limited range of responses. The home cage activity monitoring scale used in the present study was recently developed. HCA measurements could be improved in future studies by including an index of interrater agreement, conducting inter-item correlations with HCA and open field variables, and validating the instrument across studies. A 24-hour monitoring device would be useful to detect changes in physical activity in response to stress.

Body weight in the present study was a dichotomous variable: obese or non-obese. It would be interesting to examine differences in stress responses using

body weight as a continuous variable. It may be that stress responses have a linear relationship or some other predictable pattern associated with incremental increases in excess body weight. For example, epidemiological studies indicate that mortality risks are similar for individuals in the normal and overweight categories, but are significantly elevated for individuals in the obese category (Flegal et al., 2005). It is unclear whether stress responses have a similar effect based on body weight.

Obese animals in the present experiments had excess body weight for the entire experiment. In humans, it is likely that obese individuals would experience some weight fluctuations. Many obese individuals will successfully lose weight, regain lost weight, or continue to gain weight in excess of their current weight. It would be interesting to examine the effect of weight fluctuations on stress responses.

Although both experiments were well-powered to detect main effects and robust interactions, neither experiment had the statistical power to detect moderate to small interactions. This lack of power may have limited the detection of Body type X Stress interactions in Experiment I, and sex differences, Body type X Sex, Sex X Stress, and Sex X Stress X Body type interactions in Experiment II.

Future experiments should examine effects of stress in weight-stable adults to avoid changes in energy balance that accompany dynamic growth phases. The inclusion of cognitive variables that are done independent of physical activity would be valuable.

POTENTIAL CLINICAL APPLICATIONS

There are several potential clinical applications based on the findings from this project. First, if being obese results in lack of changes in behavioral responses to stressors, then it is extremely important to help obese individuals learn to modify their behaviors, especially activity behaviors, during and after stressful experiences. Studies of behavior change in obese individuals suggest that motivating people to engage in more physical activity and sustain new activity levels is not easy (Byrne, 2002; Garner & Wooley, 1991; Perri, 1998). The findings from these experiments suggest that part of the difficulty in stressful as well as non-stressful circumstances may be biologically-driven. It would be important to thoroughly understand the biological and psychological barriers to increased activity in obese individuals to best help them to modify their behaviors in health-promoting ways. The possibility that one barrier is biologically-driven rather than psychological in nature also is important for clinicians to understand when treating obese individuals who have difficulty complying with exercise regimens.

The experimental findings also highlight the potential importance of support in Post-stressor periods. These studies indicated that both lean and obese animals continued to exhibit behavioral and biological changes in the Post-stressor period, some of which could be interpreted as health-harming. If these findings extrapolate to people, then it is important to continue care and support once a stressor ceases. It is known that booster sessions and periodic telephone contact with individuals previously hospitalized for suicidal attempts help to prevent future hospitalizations

(Appleby et al., 1999). The data from the present experiments suggest that post-stressor care may also be important in the context of mild, repeated, daily stress. In particular, obese animals were relatively behaviorally unresponsive to the stressful experience while it was ongoing.

Experiment I, in particular, emphasized the impact of food access on feeding responses during stressors. In Experiment I, animals fed the cafeteria diet had access to unhealthy foods. There are two implications from these findings. First, the data suggest that if obese individuals only have access to unhealthy foods, then these foods will be consumed during stressful periods. This limited food environment is similar to the food environment typical of many lower socioeconomic neighborhoods with high levels of obesity in which the only food source is a corner store that stocks unhealthy snack foods and does not offer fresh fruit and vegetables. During periods of stress, limited access to unhealthy food types – a form of stimulus control – may be an important component of promoting or maintaining healthy eating patterns, especially among obese individuals. In addition, in Experiment I, the cafeteria diet was associated with impaired attentional processing – suggesting that consumption of these food types also may negatively affect higher cognitive processes. If this phenomenon generalizes to people, then it may be that consumption of unhealthy snack foods further compromises the ability to make healthy food choices and, in turn, may make it more difficult to maintain the motivation to engage in physical activity. The result is a negative cycle of decreased activity and poor food choices.

SUMMARY AND CONCLUSIONS

In summary, the genetic model was useful to examine obesity in rodents. Obese rats showed little activity changes but increased consumption of high caloric foods in response to mild, repeated acute stress. The relatively inert physical activity responses and substantial shift toward high caloric foods among obese rats are inconsistent with the fight or flight response to stress. The failure to alter behavioral responses to compensate for the biological responses to stress is considered maladaptive. The interpretation of these findings is that obesity is associated with maladaptive stress responses. Future studies in humans are warranted to examine the effects of stress in lean and obese individuals.

SECTION IV: FIGURES, TABLES, AND REFERENCES

APPENDIX A: FIGURES AND TABLES

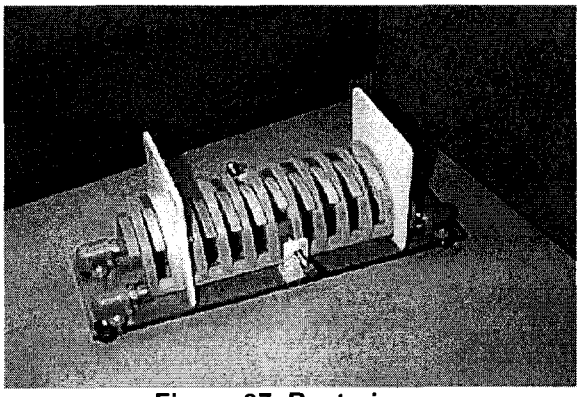

Figure 37. Restrainer.

Home Cage Activity – Version II (HCA-II)

Directions: Complete Parts A and B for each condition <u>two</u> times.

Time 1 (first 30-s interval)

A. Level of Activity

1	2	3	4	5	6	7
None	Some low	Cnst low	Some mod	Cnst mod	Some high	Cnst high

Enter Subject # and Activity rating for each subject in the group. Rating below should correspond to arrangement on housing rack. **For example: (Subject) <u># 404</u> : (Rating) <u>4</u>.**

#_____ : _____	# _____ : _____	# _____ : _____	# _____ : _____
# _____ : _____	# _____ : _____	# _____ : _____	# _____ : _____

B. Record the number of subjects <u>in this condition</u> that are engaged in the following behaviors at the end of the observation period.

Eating	Grooming	Awake/not moving	Moving HZ	Rearing	Sleeping

Time 2 (second 30-s interval)

A.

#_____ : _____	# _____ : _____	# _____ : _____	# _____ : _____
# _____ : _____	# _____ : _____	# _____ : _____	# _____ : _____

B.

Eating	Grooming	Awake/not moving	Moving HZ	Rearing	Sleeping

Figure 38. Home Cage Activity.

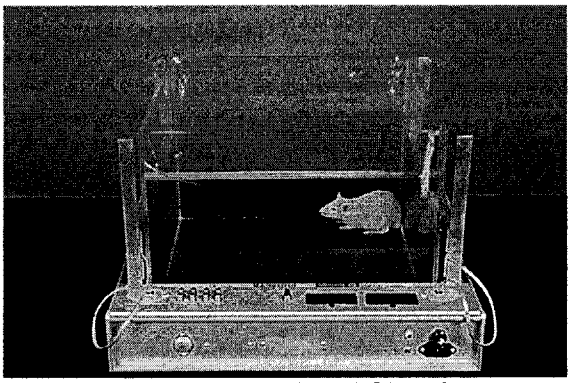

Figure 39. Open Field Chamber.

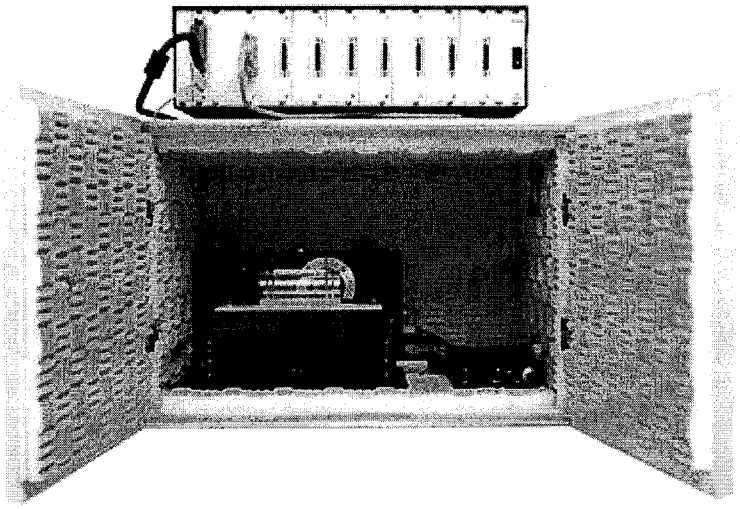

Figure 40. Acoustic Startle Response Chamber.

Table 1. Experiment I Timeline.

Date	Day of Study	Phase	Day of Phase	Measure/Activity
5 Sep 06	Day 1			Rats arrive
6 Sep 06	Day 2	**Acclimation**	Day 1	Gentle
7 Sep 06	Day 3		Day 2	Gentle
8 Sep 06	Day 4		Day 3	Gentle, FC, BW, LI
9 Sep 06	Day 5		Day 4	
10 Sep 06	Day 6		Day 5	
11 Sep 06	Day 7		Day 6	OF
12 Sep 06	Day 8		Day 7	FC, BW
13 Sep 06	Day 9		Day 8	ASR
14 Sep 06	Day 10		Day 9	Start cafeteria diet
15 Sep 06	Day 11		Day 10	FC, BW, HCA
16 Sep 06	Day 12		Day 11	
17 Sep 06	Day 13		Day 12	
18 Sep 06	Day 14		Day 13	ASR
19 Sep 06	Day 15		Day 14	FC, BW, HCA
20 Sep 06	Day 16		Day 15	OF
21 Sep 06	Day 17		Day 16	ASR
22 Sep 06	Day 18	**Baseline**	Day 1	FC, BW
23 Sep 06	Day 19		Day 2	
24 Sep 06	Day 20		Day 3	
25 Sep 06	Day 21		Day 4	FC, BW, HCA
26 Sep 06	Day 22		Day 5	
27 Sep 06	Day 23		Day 6	ASR
28 Sep 06	Day 24		Day 7	FC, BW
29 Sep 06	Day 25		Day 8	OF
30 Sep 06	Day 26		Day 9	
1 Oct 06	Day 27		Day 10	FC, BW
2 Oct 06	Day 28	**Stress**	Day 1	OF
3 Oct 06	Day 29		Day 2	HP
4 Oct 06	Day 30		Day 3	FC, BW, ASR
5 Oct 06	Day 31		Day 4	
6 Oct 06	Day 32		Day 5	FC, BW
7 Oct 06	Day 33		Day 6	OF
8 Oct 06	Day 34		Day 7	FC, BW, HP
9 Oct 06	Day 35		Day 8	
10 Oct 06	Day 36		Day 9	FC, BW, HCA
11 Oct 06	Day 37		Day 10	ASR
12 Oct 06	Day 38		Day 11	FC, BW
13 Oct 06	Day 39		Day 12	OF
14 Oct 06	Day 40		Day 13	FC, BW
15 Oct 06	Day 41		Day 14	OF
16 Oct 06	Day 42		Day 15	FC, BW, ASR
17 Oct 06	Day 43		Day 16	EPM (n = 10)
18 Oct 06	Day 44		Day 17	FC, BW, EPM (n = 30)
19 Oct 06	Day 45	**Post-stress**	Day 1	
20 Oct 06	Day 46		Day 2	ASR

Table 1. Experiment I Timeline (continued).

Date	Day of Study	Phase	Day of Phase	Measure/Activity
21 Oct 06	Day 47	**Post-stress**	Day 3	
22 Oct 06	Day 48		Day 4	
23 Oct 06	Day 49		Day 5	FC, BW, OF
24 Oct 06	Day 50		Day 6	
25 Oct 06	Day 51		Day 7	FC, BW, OF
26 Oct 06	Day 52		Day 8	ASR
27 Oct 06	Day 53		Day 9	FC, BW
28 Oct 06	Day 54		Day 10	
29 Oct 06	Day 55		Day 11	
30 Oct 06	Day 56		Day 12	FC, BW, HCA
31 Oct 06	Day 57		Day 13	
1 Nov 06	Day 58		Day 14	Stress (n = 20) FC, BW, LI, Sacrifice

FC = food consumption
BW = body weight
LI = Lee Index
OF = open field locomotor activity
ASR = acoustic startle response
HCA = home cage activity
HP = hot plate

EXPERIMENT I: FIGURES AND TABLES

Table 2. Descriptives for Body Weight (g).

Day	BD10		SD13		SD15		PD7		PD9	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
ZB	347.2	23.2	443.3	26.3	456.5	29.7	515.5	31.7	526.1	34.0
ZC	365.8	21.5	471.2	27.4	483.8	27.8	549.9	32.8	562.6	33.0
SB	234.2	17.8	302.6	24.1	307.8	23.5	340.1	26.5	347.4	24.8
SC	220.8	11.9	287.1	15.6	293.9	15.1	336.0	18.4	343.7	18.7

Table 3. Descriptives for Corticosterone (ng/mL).

Group	Re-Stress	Mean	Std		Re-Stress	Mean	Std
ZB	yes	194.4	46.0	SB	yes	380.3	191.5
	no	126.0	41.6		no	176.8	86.0
ZC	yes	280.5	30.9	SC	yes	412.3	195.9
	no	141.6	65.3		no	238.8	156.1

Table 4. Descriptives for Standard Chow Consumption (g).

Day	BD10		SD13		SD15		PD7		PD9	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
ZB	141.9	14.2	67.9	6.2	70.1	5.5	70.3	5.8	67.6	7.5
ZC	109.6	12.6	33.1	13.6	43.6	8.9	45.8	10.5	42.1	11.7
SB	97.9	6.3	44.7	4.1	51.9	4.1	51.8	3.4	50.4	4.9
SC	56.6	18.3	16.9	5.9	20.6	5.3	25.5	6.6	24.5	4.5

Table 5. Descriptives for Oreo Cookie Consumption (g).

Day	Baseline		Stress		Post	
Group	Mean	Std	Mean	Std	Mean	Std
ZC	25.4	1.4	21.4	2.7	21.4	1.5
SC	22.0	3.3	15.5	3.2	20.5	2.2

Table 6. Descriptives for Potato Chip Consumption (g).

Day	Baseline		Stress		Post	
Group	Mean	Std	Mean	Std	Mean	Std
ZC	12.3	3.2	10.4	6.7	11.4	3.8
SC	12.3	3.1	8.1	4.5	12.2	2.2

Table 7. Descriptives for Home Cage Activity Levels.

Group	Session	Mean	Std
ZB	Baseline Day	2.175	0.5534
	Stress Day	1.825	0.4257
	Post-stress Day	1.700	0.2838
ZC	Baseline Day	1.750	0.4249
	Stress Day	1.775	0.3810
	Post-stress Day	1.750	0.3909
SB	Baseline Day	2.250	0.5137
	Stress Day	2.500	0.2887
	Post-stress Day	2.675	0.2058
SC	Baseline Day	2.275	0.6061
	Stress Day	2.475	0.2486
	Post-stress Day	2.925	0.3129

Table 8. Descriptives for Open Field Locomotor during Baseline Phase.

Variable	Horizontal Activity		Vertical Activity		Center Distance		Center Time		Total Distance	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
ZB	8258.3	2029.1	529.1	412.6	1139.5	582.7	844.3	417.3	2498.3	887.1
ZC	8497.3	1134.8	639.1	181.0	1159.4	283.1	905.5	387.8	2408.6	467.5
SB	19594.4	3925.9	1540.4	475.9	3716.1	1203.7	619.6	137.4	9917.2	2843.3
SC	19775.1	5969.0	1416.9	396.7	3975.1	1428.8	691.5	275.0	10509.5	3994.2

Table 9. Descriptives for Open Field Locomotor during Stress Phase.

Variable	Horizontal Activity		Vertical Activity		Center Distance		Center Time		Total Distance	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
ZB	8132.8	1619.8	564.1	361.1	1263.1	563.8	839.0	398.6	2725.2	856.7
ZC	8457.0	1288.6	756.8	400.1	1219.6	336.8	872.0	408.0	2523.3	445.1
SB	16464.0	3672.8	1637.4	637.9	3728.8	1235.3	593.8	258.6	9201.4	2274.4
SC	17611.7	4651.3	1545.3	434.3	4004.2	1142.1	607.1	234.1	10458.1	3565.6

Table 10. Descriptives for Open Field Locomotor during Post-stress Phase.

Variable	Horizontal Activity		Vertical Activity		Center Distance		Center Time		Total Distance	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
ZB	7114.9	1794.2	604.7	424.3	1142.2	574.8	945.6	687.7	2295.8	825.2
ZC	6309.5	2218.1	671.4	414.1	862.7	462.9	943.0	359.1	1666.6	677.0
SB	18669.7	3619.2	2684.3	1118.1	5716.3	1559.2	1105.4	446.9	11594.9	2190.1
SC	19008.2	3481.5	2186.5	372.7	5754.1	1165.8	891.1	304.7	11610.6	3432.9

Table 11. Descriptives for ASR and %PPI.

Group	Stimulus	Pre-pulse	Baseline Phase Day 6		Stress Phase Day 15		Post-stress Phase Day 2	
			Mean	Std	Mean	Std	Mean	Std
ZB	110 dB	none	116.5	63.6	147.9	122.2	103.9	95.9
	110 dB	68 dB	101.1	52.4	213.9	164.6	84.5	65.6
	110 dB	82 dB	75.1	40.0	98.4	101.5	42.6	26.6
	120 dB	none	188.5	69.0	60.3	67.2	220.4	116.8
	120 dB	68 dB	150.0	65.1	145.0	107.9	113.6	69.1
	120 dB	82 dB	134.5	52.3	87.7	87.8	61.2	49.7
	Average %PPI		20.1	14.6	44.9	20.6	35.3	34.2
ZC	110 dB	none	161.6	70.7	114.7	66.7	165.1	158.0
	110 dB	68 dB	161.6	92.6	93.5	61.7	163.7	111.2
	110 dB	82 dB	69.0	31.5	62.0	60.8	72.8	105.7
	120 dB	none	240.5	76.3	185.0	89.8	223.0	104.5
	120 dB	68 dB	161.6	56.1	122.2	66.0	188.7	115.4
	120 dB	82 dB	107.1	55.2	110.0	111.0	146.4	136.1
	Average %PPI		35.5	13.8	31.2	22.4	7.1	54.0
SB	110 dB	none	156.0	86.4	329.0	147.8	265.2	178.6
	110 dB	68 dB	132.1	76.7	235.0	145.1	157.5	162.3
	110 dB	82 dB	134.7	98.1	138.0	69.6	148.9	115.8
	120 dB	none	224.7	93.5	385.9	145.2	458.9	187.7
	120 dB	68 dB	158.2	81.1	265.9	158.0	221.1	140.7
	120 dB	82 dB	195.0	128.7	224.4	117.8	193.4	127.2
	Average %PPI		17.6	27.4	39.1	21.9	38.5	39.1
SC	110 dB	none	184.4	53.7	256.5	90.0	252.8	99.0
	110 dB	68 dB	179.2	68.4	220.7	97.4	377.0	177.1
	110 dB	82 dB	127.9	30.4	185.6	66.3	272.4	157.5
	120 dB	none	250.7	90.5	353.9	81.4	520.6	164.2
	120 dB	68 dB	228.1	82.5	282.6	68.7	460.3	80.7
	120 dB	82 dB	202.2	76.9	328.0	158.5	310.5	64.0
	Average %PPI		13.1	20.1	16.7	17.8	-12.1	34.8

Table 12. ANOVA for Mean Body Weight during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	166268.130	1	166268.130	455.934	.000	.927	1.000
DIET	66.306	1	66.306	.182	.672	.005	.070
STRAIN X DIET	2568.006	1	2568.006	7.042	.012	.164	.733
Error	13128.341	36	364.676				

Table 13. ANOVA for Mean Body Weight during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	4813.636	1	4813.636	26.562	.000	.425	.999
DIET	4137.156	1	4137.156	22.829	.000	.388	.996
STRAIN X DIET	18267.076	1	18267.076	100.798	.000	.737	1.000
Error	6524.088	36	181.225				

Table 14. ANOVA for Mean Body Weight during Post-stress Phase.

Tests of Between-Subjects Effects								
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	387056.439	1	387056.439	492.148	.000	.932	492.148	1.000
DIET	2494.031	1	2494.031	3.171	.083	.081	3.171	.410
STRAIN X DIET	3872.040	1	3872.040	4.923	.033	.120	4.923	.579
Error	28312.706	36	786.464					

Table 15. ANCOVA for Mean Body Weight during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	17680.265	1	17680.265	184.417	.000	.840	1.000
STRAIN	192.263	1	192.263	2.005	.166	.054	.281
DIET	119.407	1	119.407	1.245	.272	.034	.192
STRAIN X DIET	52.724	1	52.724	.550	.463	.015	.111
Error	3355.495	35	95.871				

Table 16. ANCOVA for Mean Body Weight during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	19705.984	1	19705.984	80.136	.000	.696	1.000
STRAIN	1099.349	1	1099.349	4.471	.042	.113	.538
DIET	1589.090	1	1589.090	6.462	.016	.156	.696
STRAIN X DIET	.016	1	.016	.000	.994	.000	.050
Error	8606.722	35					

Table 17. Repeated-measures ANOVA on Body Weight within Stress Phase.

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	276511.780	7	39501.683	1954.317	.000	.982	13680.216	1.000
TIME * STRAIN	14742.814	7	2106.116	104.199	.000	.743	729.390	1.000
TIME * DIET	254.834	7	36.405	1.801	.087	.048	12.608	.721
TIME * STRAIN * DIET	247.032	7	35.290	1.746	.099	.046	12.222	.705
Error(TIME)	5093.558	252	20.213					

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	1894862.970	1	1894862.970	482.003	.000	.931	482.003	1.000
DIET	2218.145	1	2218.145	.564	.457	.015	.564	.113
STRAIN X DIET	27984.551	1	27984.551	7.119	.011	.165	7.119	.738
Error	141524.239	36	3931.229					

Table 18. Repeated-measures ANOVA on Body Weight within Post-stress Phase.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	107716.882	5	21543.376	450.295	.000	.926	2251.474	1.000
TIME * STRAIN	3927.030	5	785.406	16.416	.000	.313	82.082	1.000
TIME * DIET	331.287	5	66.257	1.385	.232	.037	6.924	.482
TIME * STRAIN * DIET	311.878	5	62.376	1.304	.264	.035	6.519	.455
Error(TIME)	8611.709	180	47.843					

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	2305654.676	1	2305654.676	501.063	.000	.933	501.063	1.000
DIET	11296.280	1	11296.280	2.455	.126	.064	2.455	.332
STRAIN X DIET	22716.515	1	22716.515	4.937	.033	.121	4.937	.580
Error	165654.863	36	4601.524					

Table 19. Repeated-measures ANCOVA on Body Weight within Stress Phase

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	195.244	7	83.828	1.480	.232	.041	.332
TIME X BASELINE BW	476.032	7	204.383	3.608	.025	.093	.699
TIME X STRAIN	222.841	7	95.676	1.689	.186	.046	.374
TIME X DIET	215.667	7	92.596	1.635	.196	.045	.363
TIME X STRAIN DIET	91.148	7	39.134	.691	.525	.019	.172
Error(TIME)		245	56.643				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	126610.739	1	126610.739	297.139	.000	.895	1.000
STRAIN	889.439	1	889.439	2.087	.157	.056	.290
DIET	473.268	1	473.268	1.111	.299	.031	.176
STRAIN X DIET	82.199	1	82.199	.193	.663	.005	.071
Error	14913.501	35	426.100				

Table 20. Repeated-measures ANCOVA on Body Weight within Post-stress Phase

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	104.920	5	34.322	.441	.728	.012	.137
TIME X BASELINE BW	276.348	5	90.401	1.160	.329	.032	.308
TIME X STRAIN	141.583	5	46.315	.595	.623	.017	.171
TIME X DIET	300.609	5	98.337	1.262	.291	.035	.333
TIME X STRAIN DIET	407.769	5	133.392	1.712	.168	.047	.441
Error(TIME)	8335.360	175	77.906				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	118575.935	1	118575.935	88.153	.000	.716	1.000
STRAIN	6281.703	1	6281.703	4.670	.038	.118	.556
DIET	6659.551	1	6659.551	4.951	.033	.124	.581
STRAIN X DIET	2.080	1	2.080	.002	.969	.000	.050
Error	47078.928	35	1345.112				

Table 21. Repeated-measures ANOVA on Mean Body Weight during Stress and Post-stress Phases.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	70567.200	1	70567.200	1467.367	.000	.976	1467.367	1.000
TIME X STRAIN	4770.961	1	4770.961	99.207	.000	.734	99.207	1.000
TIME X DIET	436.178	1	436.178	9.070	.005	.201	9.070	.834
TIME X STRAIN DIET	10.224	1	10.224	.213	.648	.006	.213	.073
Error(TIME)	1731.277	36	48.091					

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	657339.411	1	657339.411	496.968	.000	.932	496.968	1.000
DIET	2474.200	1	2474.200	1.871	.180	.049	1.871	.265
STRAIN X DIET	8317.081	1	8317.081	6.288	.017	.149	6.288	.684
Error	47617.190	36	1322.700					

Table 22. Repeated-measures ANCOVA for Mean Body Weight during Stress and Post-stress Phases.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	125.894	1	125.894	2.586	.117	0.069	0.346
TIME X BASELINE BW	27.460	1	27.460	0.564	.458	0.016	0.113
TIME X STRAIN	186.062	1	186.062	3.822	.059	0.098	0.477
TIME X DIET	418.647	1	418.647	8.600	.006	0.197	0.814
TIME X STRAIN DIET	25.441	1	25.441	0.523	.475	0.015	0.108
Error(TIME)	1703.817	35	48.680				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	37358.789	1	37358.789	127.462	.000	.785	1.000
STRAIN	1105.551	1	1105.551	3.772	.060	.097	.472
DIET	1289.850	1	1289.850	4.401	.043	.112	.532
STRAIN X DIET	27.300	1	27.300	0.093	.762	.003	.060
Error	10258.400	35	293.097				

Table 23. ANOVA for Lee Index.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	42627811536445.80	1	42627811536445.80	480.213	.000	.930	1.000
DIET	194314612149.52	1	194314612149.52	2.189	.148	.057	.302
STRAIN X DIET	400402520813.47	1	400402520813.47	4.511	.041	.111	.543
Error	3195668858774.37	36	88768579410.40				

Table 24. ANOVA for Corticosterone by Strain and Diet.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	135547.573	1	135547.573	6.949	.012	.162	.727
DIET	23915.230	1	23915.230	1.226	.276	.033	.190
STRAIN X DIET	36.895	1	36.895	0.002	.966	.000	.050
Error	702226.517	36	19506.292				

Table 25. ANOVA for Corticosterone by Strain, Diet, and Re-exposure to Stress.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	135547.573	1	135547.573	9.359	.004	.226	.843
DIET	23915.230	1	23915.230	1.651	.208	.049	.238
RE-STRESS	213378.180	1	213378.180	14.733	.001	.315	.961
STRAIN X DIET	36.895	1	36.895	0.003	.960	.000	.050
STRAIN X RE-STRESS	18021.978	1	18021.978	1.244	.273	.037	.191
DIET X RE-STRESS	1026.291	1	1026.291	0.071	.792	.002	.058
STRAIN X DIET X RE-STRESS	6328.646	1	6328.646	0.437	.513	.013	.098
Error	463471.423	32	14483.482				

Table 26. ANOVA for Standard Food Consumption on Baseline Day 10

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	6545.922	1	6545.922	232.397	.000	.866	1.000
DIET	3886.812	1	3886.812	137.992	.000	.793	1.000
STRAIN X DIET	129.240	1	129.240	4.588	.039	.113	.550
Error	1014.013	36	28.167				

Table 27. ANOVA for Mean Standard Food Consumption on Stress Days 13 and 15

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE CHOW	118.650	1	118.650	10.631	.002	.233	.887
STRAIN	0.994	1	0.994	0.089	.767	.003	.060
DIET	136.041	1	136.041	12.190	.001	.258	.924
STRAIN X DIET	99.969	1	99.969	8.957	.005	.204	.829
Error	390.613	35	11.160				

Table 28. ANCOVA for Mean Standard Food Consumption on Post-stress Days 7 and 9

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE CHOW	110.254	1	110.254	13.874	.001	.284	.952
STRAIN	0.782	1	0.782	0.098	.756	.003	.061
DIET	80.929	1	80.929	10.184	.003	.225	.873
STRAIN X DIET	7.596	1	7.596	0.956	.335	.027	.158
Error	278.134	35	7.947				

Table 29. Repeated-measures ANCOVA on Standard Chow Food Consumption during Stress Phase

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	272.055	7	38.865	2.566	.014	.068	.883
TIME X CHOW	211.346	7	30.192	1.993	.057	.054	.773
TIME X STRAIN	344.614	7	49.231	3.250	.003	.085	.953
TIME X DIET	131.026	7	18.718	1.236	.284	.034	.526
TIME X STRAIN DIET	247.977	7	35.425	2.339	.025	.063	.847
Error(TIME)	3710.958	245	15.147				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
CHOW	567.754	1	567.754	12.039	.001	.256	.921
STRAIN	149.053	1	149.053	3.160	.084	.083	.409
DIET	1254.107	1	1254.107	26.592	.000	.432	.999
STRAIN X DIET	272.362	1	272.362	5.775	.022	.142	.647
Error	1650.649	35	47.161				

Table 30. Repeated-measures ANCOVA on Standard Chow Food Consumption during Post-stress Phase

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	25.835	3	8.612	1.707	.170	.047	.435
TIME X CHOW	27.807	3	9.269	1.837	.145	.050	.465
TIME X STRAIN	55.795	3	18.598	3.687	.014	.095	.790
TIME X DIET	13.676	3	4.559	0.904	.442	.025	.242
TIME X STRAIN DIET	45.849	3	15.283	3.029	.033	.080	.698
Error(TIME)	529.723	105	5.045				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
CHOW	455.132	1	455.132	16.369	.000	.319	.976
STRAIN	22.182	1	22.182	0.798	.378	.022	.140
DIET	228.519	1	228.519	8.219	.007	.190	.796
STRAIN X DIET	0.230	1	0.230	0.008	.928	.000	.051
Error	973.139	35	27.804				

Table 31. Repeated-measures ANCOVA on Standard Chow Food Consumption during Stress and Post-stress Phases.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	0.070	1	0.070	0.016	.900	.000	.052
TIME X CHOW	0.077	1	0.077	0.018	.895	.001	.052
TIME X STRAIN	0.006	1	0.006	0.001	.970	.000	.050
TIME X DIET	3.558	1	3.558	0.811	.374	.023	.142
TIME X STRAIN DIET	26.226	1	26.226	5.980	.020	.146	.662
Error(TIME)	153.504	35	4.386				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE CHOW	228.827	1	228.827	15.544	.000	.308	.969
STRAIN	1.769	1	1.769	0.120	.731	.003	.063
DIET	213.413	1	213.413	14.497	.001	.293	.959
STRAIN X DIET	81.339	1	81.339	5.525	.025	.136	.628
Error	515.243	35	14.721				

Table 32. ANOVA for Chip Consumption during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	4.418	1	4.418	1.104	.307	.058	.169
Error	72.002	18	4.000				

Table 33. ANOVA for Chip Consumption during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	70.125	1	70.125	16.750	.001	.482	.972
Error	75.357	18	4.187				

Table 34. ANOVA for Chip Consumption during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	0.861	1	0.861	0.254	.620	.014	.077
Error	61.040	18	3.391				

Table 35. Repeated-measures ANOVA for Chip Consumption during Baseline, Stress, and Post-stress Phases.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	10.461	2	5.231	1.710	.195	.087	.336
TIME X STRAIN	65.884	2	32.942	10.768	.000	.374	.984
Error(TIME)	110.135	36	3.059				

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	9.520	1	9.520	1.744	.203	0.088	0.240
Error	98.265	18	5.459				

Table 36. ANOVA for Cookie Consumption during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	0.001	1	0.001	0.001	.981	.000	.050
Error	15.885	18	0.883				

Table 37. ANOVA for Cookie Consumption during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	27.495	1	27.495	8.073	.011	.310	.766
Error	61.307	18	3.406				

Table 38. ANOVA for Cookie Consumption during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	3.200	1	3.200	1.404	.251	.072	.202
Error	41.017	18	2.279				

Table 39. Repeated-measures ANOVA for Cookie Consumption during Baseline, Stress, and Post-stress Phases.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	82.916	2	41.458	21.004	.000	0.539	1.000
TIME X STRAIN	14.315	2	7.158	3.626	.037	0.168	.633
Error(TIME)	71.056	36	1.974				

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	16.380	1	16.380	6.253	.022	.258	.658
Error	47.153	18	2.620				

Table 40. ANCOVA for Total Kilocalories during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	4813.636	1	4813.636	26.562	.000	.425	.999
DIET	4137.156	1	4137.156	22.829	.000	.388	.996
STRAIN X DIET	18267.076	1	18267.076	100.798	.000	.737	1.000
Error	181.225	36	181.225				

Table 41. ANCOVA for Total Kilocalories during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE KCAL	306.857	1	306.857	2.846	.100	.075	.375
STRAIN	8811.567	1	8811.567	81.736	.000	.700	1.000
DIET	8537.319	1	8537.319	79.192	.000	.693	1.000
STRAIN X DIET	917.222	1	917.222	8.508	.006	.196	.809
Error	3773.177	35	107.805				

Table 42. ANCOVA for Total Kilocalories during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE KCAL	327.476	1	327.476	1.352	.253	.037	.205
STRAIN	3376.197	1	3376.197	26.063	.000	.427	.999
DIET	10628.507	1	10628.507	82.048	.000	.701	1.000
STRAIN X DIET	191.072	1	191.072	1.475	.233	.040	.219
Error	4533.891	35	129.540				

Table 43. Repeated-measures ANOVA on Total Kilocalories Consumed during Stress Phase

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	9136.606	7	2448.943	1.361	.253	.037	.400
TIME X KCAL	7362.642	7	1973.456	1.097	.359	.030	.326
TIME X STRAIN	7572.170	7	2029.627	1.128	.345	.031	.334
TIME X DIET	5320.681	7	1426.136	.793	.524	.022	.240
TIME X STRAIN DIET	10042.702	7	2132.850	1.496	.210	.041	.437
Error(TIME)	234910.126	245	6711.718				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
KCAL	22295.133	1	22295.133	10.761	.005	.235	.891
STRAIN	16725.688	1	16725.688	8.073	.002	.187	.789
DIET	54118.634	1	54118.634	26.122	.007	.427	.999
STRAIN X DIET	2895.922	1	2895.922	1.398	.000	.038	.210
Error	72512.434	35	2071.784				

Table 44. Repeated-measures ANOVA on Total Kilocalories Consumed during Post-stress Phase

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	15842.506	3	5280.835	5.020	.003	.125	.907
TIME X KCAL	15355.936	3	5118.645	4.866	.003	.122	.898
TIME X STRAIN	24136.913	3	8045.638	7.648	.000	.179	.985
TIME X DIET	6832.393	3	2277.464	2.165	.097	.058	.537
TIME X STRAIN DIET	4317.292	3	1439.097	1.368	.257	.038	.355
Error(TIME)	110460.914	105	1052.009				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
KCAL	25436.130	1	25436.130	8.058	.007	.187	.788
STRAIN	8732.506	1	8732.506	2.766	.105	.073	.366
DIET	31155.43	1	31155.43	9.869	.003	.220	.863
STRAIN X DIET	3666.368	1	3666.368	1.161	.289	.032	.182
Error	110488.798	35	3156.823				

Table 45. Repeated-measures ANCOVA on Total Kilocalories during Stress and Post-stress Phases.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	8.228	1	8.228	.104	.749	.003	.061
TIME X KCAL	9.168	1	9.168	.116	.735	.003	.063
TIME X STRAIN	639.564	1	639.564	8.091	.007	.188	.790
TIME X DIET	57.213	1	57.213	.724	.401	.020	.131
TIME X STRAIN DIET	135.512	1	135.512	1.714	.199	.047	.247
Error(TIME)	2766.588	35	79.045				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
KCAL	634.165	1	472.864	2.987	.093	.079	.390
STRAIN	11554.444	1	11548.200	72.952	.000	.676	1.000
DIET	17669.810	1	19108.613	120.712	.000	.775	1.000
STRAIN X DIET	1241.735	1	972.782	6.145	.018	.149	.674
Error	6612.913	35	158.299				

Table 46. ANOVA for Home Cage Activity Level during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	.900	1	.900	3.220	.081	.082	.416
DIET	.400	1	.400	1.431	.239	.038	.214
STRAIN X DIET	.506	1	.506	1.811	.187	.048	.58
Error	10.063	36	.280				

Table 47. ANOVA for Home Cage Activity Level during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	4.727	1	4.727	40.096	.000	.527	1.000
DIET	.014	1	.014	.119	.732	.003	.063
STRAIN X DIET	.002	1	.002	.013	.909	.000	.013
Error	4.244	36	.118				

Table 48. ANOVA for Home Cage Activity Level during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	11.556	1	11.556	123.725	.000	.775	1.000
DIET	.225	1	.225	2.409	.129	.063	.327
STRAIN X DIET	.100	1	.100	1.071	.308	.029	.172
Error	3.363	36	.093				

Table 49. Repeated-measures for Home Cage Activity Level during Baseline, Stress, and Post-stress Phases.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	0.501	2	0.251	1.617	.206	.043	.331
TIME X STRAIN	3.003	2	1.502	9.693	.000	.212	.978
TIME X DIET	0.614	2	0.307	1.980	.145	.052	.397
TIME X STRAIN DIET	0.228	2	0.114	0.736	.482	.020	.170
Error(TIME)	11.154	72	0.155				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	14.180	1	14.180	78.358	.000	.685	1.000
DIET	0.026	1	0.026	0.141	.709	.004	.065
STRAIN X DIET	0.380	1	0.380	2.098	.156	.055	.292
Error	6.515	36	0.181				

Table 50. Chi-Square for Home Cage Behaviors during Baseline Phase.

Dependent Variable and (df)	Condition	N	Mean Rank
Eating Strain: χ^2 (1) = 0.046, p = ns Diet: χ^2 (1) = 1.156, p = ns	Zucker	20	8.75
	Sprague-Dawley	20	8.25
	Bland	20	9.75
	Cafeteria	20	7.25
Grooming Strain: χ^2 (1) = 5.503, p = 0.019 Diet: χ^2 (1) = 0.298, p = ns	Zucker	20	5.81
	Sprague-Dawley	20	11.19
	Bland	20	7.88
	Cafeteria	20	9.13
Awake/not moving Strain: χ^2 (1) = 3.293, p = 0.070 Diet: χ^2 (1) = 1.742, p = ns	Zucker	20	10.56
	Sprague-Dawley	20	6.44
	Bland	20	7.00
	Cafeteria	20	10.00
Horizontal Activity Strain: χ^2 (1) = 1.057, p = ns Diet: χ^2 (1) = 0.470, p = ns	Zucker	20	7.38
	Sprague-Dawley	20	9.63
	Bland	20	7.75
	Cafeteria	20	9.25
Vertical Activity Strain: χ^2 (1) = 2.445, p = ns Diet: χ^2 (1) = 0.003, p = ns	Zucker	20	6.69
	Sprague-Dawley	20	10.31
	Bland	20	8.56
	Cafeteria	20	8.44
Sleeping Strain: χ^2 (1) = 0.964, p = ns Diet: χ^2 (1) = 1.714, p = ns	Zucker	20	9.63
	Sprague-Dawley	20	7.38
	Bland	20	10.00
	Cafeteria	20	7.00

Table 51. Chi-Square for Home Cage Behaviors during Stress Phase.

Dependent Variable and (df)	Condition	N	Mean Rank
Eating Strain: χ^2 (1) = 0.003, p = ns Diet: χ^2 (1) = 2.963, p = 0.085	Zucker	20	8.44
	Sprague-Dawley	20	8.56
	Bland	20	8.50
	Cafeteria	20	10.50
Grooming Strain: χ^2 (1) = 3.052, p = 0.081 Diet: χ^2 (1) = 0.429, p = ns	Zucker	20	8.50
	Sprague-Dawley	20	10.50
	Bland	20	7.75
	Cafeteria	20	9.25
Awake/not moving Strain: χ^2 (1) = 9.408, p = 0.002 Diet: χ^2 (1) = 0.108, p = ns	Zucker	20	12.00
	Sprague-Dawley	20	5.00
	Bland	20	8.13
	Cafeteria	20	8.88
Horizontal Activity Strain: χ^2 (1) = 1.507, p = ns Diet: χ^2 (1) = 0.112, p = ns	Zucker	20	7.13
	Sprague-Dawley	20	9.88
	Bland	20	8.13
	Cafeteria	20	8.88
Vertical Activity Strain: χ^2 (1) = 0.597, p = ns Diet: χ^2 (1) = 0.597, p = ns	Zucker	20	9.38
	Sprague-Dawley	20	7.63
	Bland	20	6.38
	Cafeteria	20	10.63
Sleeping Strain: χ^2 (1) = 3.210, p = 0.073 Diet: χ^2 (1) = 3.210, p = 0.073	Zucker	20	10.25
	Sprague-Dawley	20	6.75
	Bland	20	10.25
	Cafeteria	20	6.75

Table 52. Chi-Square for Home Cage Behaviors during Post-stress Phase.

Dependent Variable and (df)	Condition	N	Mean Rank
Eating Strain: χ^2 (1) = 1.682, p = ns Diet: χ^2 (1) = 0.105, p = ns	Zucker	20	10.00
	Sprague-Dawley	20	7.00
	Bland	20	8.13
	Cafeteria	20	8.88
Grooming Strain: χ^2 (1) = 2.290, p = ns Diet: χ^2 (1) = 0.105, p = ns	Zucker	20	10.25
	Sprague-Dawley	20	6.75
	Bland	20	8.88
	Cafeteria	20	8.13
Awake/not moving Strain: χ^2 (1) = 11.815, p = 0.001 Diet: χ^2 (1) = 0.565, p = ns	Zucker	20	12.50
	Sprague-Dawley	20	4.50
	Bland	20	7.63
	Cafeteria	20	9.38
Horizontal Activity Strain: χ^2 (1) = 1.023, p = ns Diet: χ^2 (1) = 0.114, p = ns	Zucker	20	7.38
	Sprague-Dawley	20	9.63
	Bland	20	8.88
	Cafeteria	20	8.13
Vertical Activity Strain: χ^2 (1) = 12.347, p < 0.0001 Diet: χ^2 (1) = 0.509, p = ns	Zucker	20	4.50
	Sprague-Dawley	20	12.50
	Bland	20	9.31
	Cafeteria	20	7.69
Sleeping Strain: χ^2 (1) = 4.923, p = 0.027 Diet: χ^2 (1) = 0.019, p = ns	Zucker	20	10.50
	Sprague-Dawley	20	6.50
	Bland	20	8.38
	Cafeteria	20	8.63

Table 53. ANOVA for Baseline Horizontal Activity.

Tests of Between-Subjects Effects								
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	1278471183.025	1	1278471183.025	90.598	.000	.716	90.598	1.000
DIET	440370.225	1	440370.225	.031	.861	.001	.031	.053
STRAIN X DIET	8497.225	1	8497.225	.001	.981	.000	.001	.050
Error	508014591.500	36	14111516.431					

Table 54. ANOVA for Baseline Vertical Activity.

Tests of Between-Subjects Effects								
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	8002197.025	1	8002197.025	54.541	.000	.602	54.541	1.000
DIET	455.625	1	455.625	.003	.956	.000	.003	.050
STRAIN X DIET	136305.625	1	136305.625	.929	.342	.025	.929	.155
Error	5281869.100	36	146718.586					

Table 55. ANCOVA for Horizontal Activity during Stress Phase.

Tests of Between-Subjects Effects								
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
BASELINE HA	94376546.335	1	94376546.335	12.690	.001	.266	12.690	.934
STRAIN	42577387.616	1	42577387.616	5.725	.022	.141	5.725	.643
DIET	4163108.920	1	4163108.920	.560	.459	.016	.560	.113
STRAIN X DIET	1800394.716	1	1800394.716	.242	.626	.007	.242	.077
Error	260302581.365	35	7437216.610					

Table 56. ANCOVA for Vertical Activity during Stress Phase.

Tests of Between-Subjects Effects								
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
BASELINE VA	1964266.923	1	1964266.923	11.438	.002	.246	11.438	.908
STRAIN	590519.008	1	590519.008	3.439	.072	.089	3.439	.438
DIET	29608.816	1	29608.816	.172	.681	.005	.172	.069
STRAIN X DIET	49422.869	1	49422.869	.288	.595	.008	.288	.082
Error	6010546.077	35	171729.888					

Table 57. ANCOVA for Horizontal Activity during Post-stress Phase.

Tests of Between-Subjects Effects								
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
BASELINE HA	18271521.321	1	18271521.321	2.268	.141	.061	2.268	.311
STRAIN	283365494.057	1	283365494.057	35.175	.000	.501	35.175	1.000
DIET	745996.522	1	745996.522	.093	.763	.003	.093	.060
STRAIN X DIET	3334755.535	1	3334755.535	.414	.524	.012	.414	.096
Error	281953153.779	35	8055804.394					

Table 58. ANCOVA for Vertical Activity during Post-stress Phase.

Tests of Between-Subjects Effects								
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
BASELINE VA	2398824.424	1	2398824.424	6.329	.017	.153	6.329	.687
STRAIN	5673200.563	1	5673200.563	14.968	.000	.300	14.968	.964
DIET	445176.117	1	445176.117	1.175	.286	.032	1.175	.184
STRAIN X DIET	403983.627	1	403983.627	1.066	.309	.030	1.066	.171
Error	13265946.676	35	379027.048					

Table 59. Repeated-measures ANCOVA on Horizontal Activity.

Tests of Within-Subjects Effects								
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	14812251.790	1	14812251.790	2.640	.113	.070	2.640	.352
TIME * BASELINE HA	14798101.225	1	14798101.225	2.638	.113	.070	2.638	.352
TIME * STRAIN	53130817.762	1	53130817.762	9.470	.004	.213	9.470	.849
TIME * DIET	4216842.362	1	4216842.362	.752	.392	.021	.752	.135
TIME * STRAIN * DIET	117294.275	1	117294.275	.021	.886	.001	.021	.052
Error(TIME)	196368311.775	35	5610523.194					

Tests of Between-Subjects Effects								
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
BASELINE HA	97849966.431	1	97849966.431	9.901	.003	.221	9.901	.864
STRAIN	272812063.911	1	272812063.911	27.606	.000	.441	27.606	.999
DIET	692263.081	1	692263.081	.070	.793	.002	.070	.058
STRAIN X DIET	5017855.976	1	5017855.976	.508	.481	.014	.508	.107
Error	345887423.369	35	9882497.811					

Table 60. Repeated-measures ANCOVA on Vertical Activity.

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	262453.685	1	262453.685	1.284	.265	.035	1.284	.197
TIME * BASELINE VA	10847.290	1	10847.290	.053	.819	.002	.053	.056
TIME * STRAIN	1301522.994	1	1301522.994	6.366	.016	.154	6.366	.689
TIME * DIET	352201.602	1	352201.602	1.723	.198	.047	1.723	.248
TIME * STRAIN * DIET	85402.045	1	85402.045	.418	.522	.012	.418	.096
Error(time)	7155233.360	35	204435.239					

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
BASELINE VA	4352244.056	1	4352244.056	12.567	.001	.264	12.567	.931
STRAIN	4962196.577	1	4962196.577	14.328	.001	.290	14.328	.957
DIET	122583.330	1	122583.330	.354	.556	.010	.354	.089
STRAIN X DIET	368004.451	1	368004.451	1.063	.310	.029	1.063	.171
Error	12121259.394	35	346321.697					

Table 61. ANOVA for 110 dB Startle during Baseline Phase.

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	9687.638	1	9687.638	1.998	.166	.053	1.998	.280
DIET	13527.294	1	13527.294	2.790	.104	.072	2.790	.369
STRAIN X DIET	704.755	1	704.755	.145	.705	.004	.145	.066
Error	174533.642	36	4848.157					

Table 62. ANOVA for 120 dB Startle during Baseline Phase.

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	5361.218	1	5361.218	.780	.383	.021	.780	.138
DIET	15223.111	1	15223.111	2.214	.145	.058	2.214	.305
STRAIN X DIET	1695.023	1	1695.023	.247	.623	.007	.247	.077
Error	247531.367	36	6875.871					

Table 63. ANOVA for Average %PPI during Baseline Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	1549.620	1	1549.620	3.965	.054	.099	.491
DIET	301.891	1	301.891	.772	.385	.021	.137
STRAIN X DIET	986.099	1	986.099	2.523	.121	.065	.340
Error	14069.586	36	390.822				

Table 64. ANOVA for 110 dB Startle during Stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	260663.075	1	260663.075	21.140	.000	.370	.994
DIET	27925.861	1	27925.861	2.265	.141	.059	.311
STRAIN X DIET	3884.591	1	3884.591	.315	.578	.009	.085
Error	443894.691	36	12330.408				

Table 65. ANOVA for 120 dB Startle during Stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	290686.337	1	290686.337	18.492	.000	.339	.987
DIET	9281.321	1	9281.321	.590	.447	.016	.116
STRAIN X DIET	24.745	1	24.745	.002	.969	.000	.050
Error	565890.799	36	15719.189				

Table 66. ANOVA for Average %PPI during Stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	1033.705	1	1033.705	2.403	.130	.063	.326
DIET	3244.258	1	3244.258	7.541	.009	.173	.762
STRAIN X DIET	187.567	1	187.567	.436	.513	.012	.099
Error	15488.529	36	430.237				

Table 67. ANOVA for 110 dB Startle during Post-stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	155062.851	1	155062.851	8.175	.007	.185	.795
DIET	5972.469	1	5972.469	.315	.578	.009	.085
STRAIN X DIET	13561.370	1	13561.370	.715	.403	.019	.131
Error	682820.616	36	18967.239				

Table 68. ANOVA for 120 dB Startle during Post-stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	718639.831	1	718639.831	33.133	.000	.479	1.000
DIET	10351.145	1	10351.145	.477	.494	.013	.103
STRAIN X DIET	8741.303	1	8741.303	.403	.530	.011	.095
Error	780817.976	36	21689.388				

Table 69. ANOVA for Average %PPI during Post-stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
STRAIN	633.996	1	633.996	.371	.546	.010	.091
DIET	15560.982	1	15560.982	9.107	.005	.202	.836
STRAIN X DIET	1263.040	1	1263.040	.739	.396	.020	.133
Error	61510.503	36	1708.625				

Table 70. Repeated-measures ANOVA on 110 dB Startle.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	70795.477	2	35397.739	3.282	.043	.084	6.564	.606
TIME * STRAIN	90239.202	2	45119.601	4.184	.019	.104	8.367	.719
TIME * DIET	47191.923	2	23595.962	2.188	.120	.057	4.376	.433
TIME * STRAIN * DIET	4097.656	2	2048.828	.190	.827	.005	.380	.078
Error(time)	776524.062	72	10785.056					

Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	335174.362	1	335174.362	22.995	.000	.390	22.995	.997
DIET	233.700	1	233.700	.016	.900	.000	.016	.052
STRAIN X DIET	14053.060	1	14053.060	.964	.333	.026	.964	.159
Error	524724.888	36	14575.691					

Table 71. Repeated-measures ANOVA for 120 dB Startle.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	337150.110	2	168575.055	15.796	.000	.305	31.592	.999
TIME * STRAIN	304056.751	2	152028.375	14.245	.000	.284	28.491	.998
TIME * DIET	29327.228	2	14663.614	1.374	.260	.037	2.748	.287
TIME * STRAIN * DIET	9713.733	2	4856.867	.455	.636	.012	.910	.122
Error(time)	768393.928	72	10672.138					

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	710630.636	1	710630.636	30.978	.000	.463	30.978	1.000
DIET	5528.348	1	5528.348	.241	.626	.007	.241	.077
STRAIN X DIET	747.337	1	747.337	.033	.858	.001	.033	.054
Error	825846.213	36	22940.173					

Table 72. Repeated-measures ANOVA on Average %PPI.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
TIME	5305.383	2	2652.691	3.195	.047	.082	6.389	.594
TIME * STRAIN	100.630	2	50.315	.061	.941	.002	.121	.059
TIME * DIET	10106.005	2	5053.002	6.085	.004	.145	12.171	.874
TIME * STRAIN * DIET	269.265	2	134.632	.162	.851	.004	.324	.074
Error(time)	59786.400	72	830.367					

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
STRAIN	3116.691	1	3116.691	3.587	.066	.091	3.587	.454
DIET	9001.126	1	9001.126	10.359	.003	.223	10.359	.879
STRAIN X DIET	2167.441	1	2167.441	2.494	.123	.065	2.494	.336
Error	31282.218	36	868.951					

Table 73. Paired t-tests Comparing Changes between Phases for Each Group on All Variables.

Group	Baseline Phase to Stress Phase Comparison	t value (df)	p value	Stress Phase to Post-stress Phase Comparison	t value (df)	p value
ZB	BW	-38.330 (9)	.000	BW	-23.617 (9)	.000
	bland food	19.97 (9)	.000	bland food	7.073 (9)	.000
	total kcal	20.071 (9)	.000	total kcal	3.122 (9)	.012
	HCA	1.397 (9)	.196	HCA	1.103 (9)	.299
	HA	0.282 (9)	.784	HA	1.585 (9)	.147
	VA	0.402 (9)	.697	VA	-0.253 (9)	.806
	CD	-1.132 (9)	.287	CD	0.698 (9)	.503
	CT	0.036 (9)	.972	CT	-0.572 (9)	.581
	110 dB	-0.817 (9)	.435	110 dB	1.442 (9)	.183
	120 dB	-0.478 (9)	.644	120 dB	-1.43 (9)	.889
	Average %PPI	-1.584 (9)	.148	Average %PPI	1.204 (9)	.259
ZC	BW	-27.283 (9)	.000	BW	-23.079 (9)	.000
	bland food	18.432 (9)	.000	bland food	-1.478 (9)	.173
	chips	-3.770 (9)	.004	chips	3.381 (9)	.008
	cookies	2.198 (9)	.056	cookies	-0.275 (9)	.790
	total kcal	-1.008 (9)	.340	total kcal	0.097 (9)	.925
	HCA	-0.287 (9)	.780	HCA	0.176 (9)	.964
	HA	0.073 (9)	.943	HA	2.536 (9)	.032
	VA	-0.931 (9)	.376	VA	0.482 (9)	.641
	CD	-0.431 (9)	.676	CD	2.102 (9)	.065
	CT	0.136 (9)	.874	CT	-0.498 (9)	.631
	110 dB	1.845 (9)	.098	110 dB	-0.931 (9)	.385
	120 dB	1.797 (9)	.106	120 dB	-0.994 (9)	.346
	Average %PPI	0.572 (9)	.581	Average %PPI	1.136 (9)	.285

Table 73. Paired t-tests Comparing Changes between Phases for Each Group on All Variables (continued).

Group	Baseline Phase to Stress Phase Comparison	t value (df)	p value	Stress Phase to Post-stress Phase Comparison	t value (df)	p value
SB	BW	-29.209 (9)	.000	BW	-10.508 (9)	.000
	bland food	26.376 (9)	.000	bland food	-0.499 (9)	.630
	total kcal	26.336 (9)	.000	total kcal	-4.723 (9)	.001
	HCA	-1.464 (9)	.177	HCA	-1.561 (9)	.153
	HA	2.545 (9)	.031	HA	-1.463 (9)	.177
	VA	-0.572 (9)	.582	VA	-3.58 (9)	.006
	CD	-0.024 (9)	.981	CD	-4.181 (9)	.002
	CT	0.33 (9)	.749	CT	-3.61 (9)	.006
	110 dB	-3.156 (9)	.012	110 dB	0.922 (9)	.380
	120 dB	-3.926 (9)	.003	120 dB	-1.162 (9)	.275
	Average %PPI	-2.317 (9)	.046	Average %PPI	0.041 (9)	.986
SC	BW	-20.832 (9)	0.000	BW	-23.708 (9)	.020
	bland food	9.474 (9)	.000	bland food	-0.035 (9)	.973
	chips	4.326 (9)	.002	chips	-1.255 (9)	.241
	cookies	6.712 (9)	.000	cookies	-3.26 (9)	.010
	total kcal	13.550 (9)	.000	total kcal	-6.946 (9)	.000
	HCA	-0.923 (9)	.380	HCA	-2.946 (9)	.016
	HA	1.315 (9)	.221	HA	-1.229 (9)	.250
	VA	-0.840 (9)	.423	VA	-4.975 (9)	.001
	CD	-0.094 (9)	.927	CD	-5.166 (9)	.001
	CT	0.999 (9)	.344	CT	-2.100 (9)	.065
	110 dB	-2.624 (9)	.028	110 dB	0.074 (9)	.942
	120 dB	-4.032 (9)	.003	120 dB	-2.763 (9)	.022
	Average %PPI	0.546 (9)	.589	Average %PPI	2.716 (9)	.024

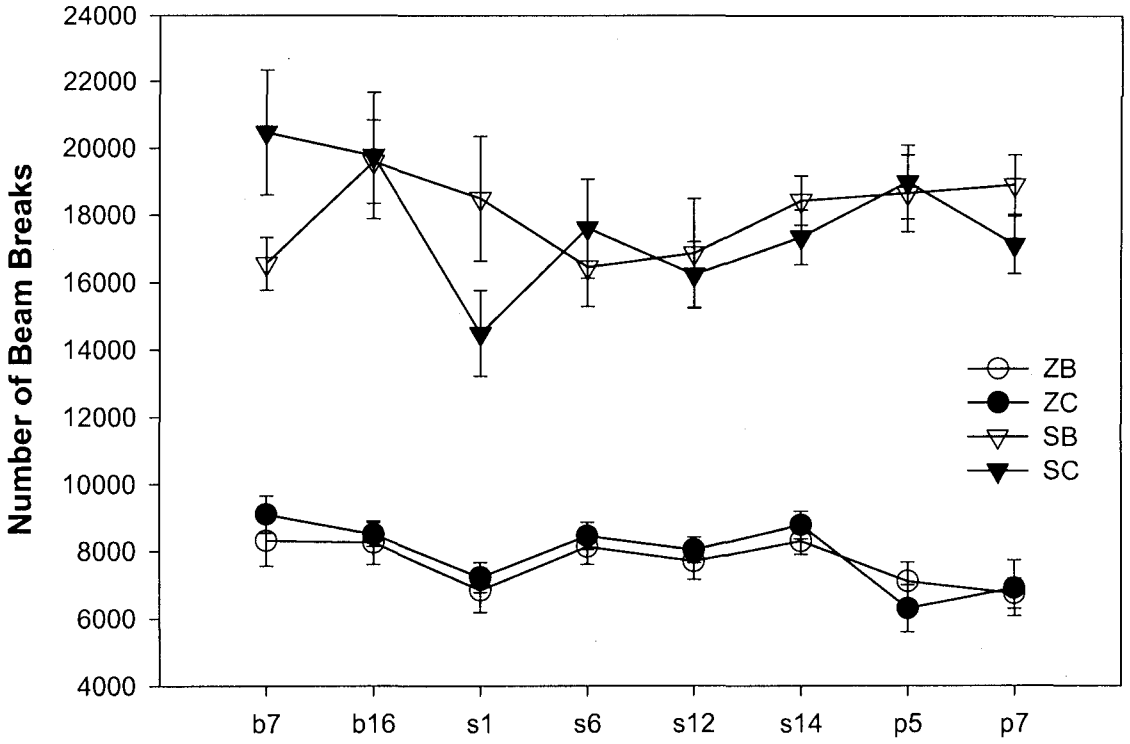

Figure 41. Horizontal activity for each testing session.

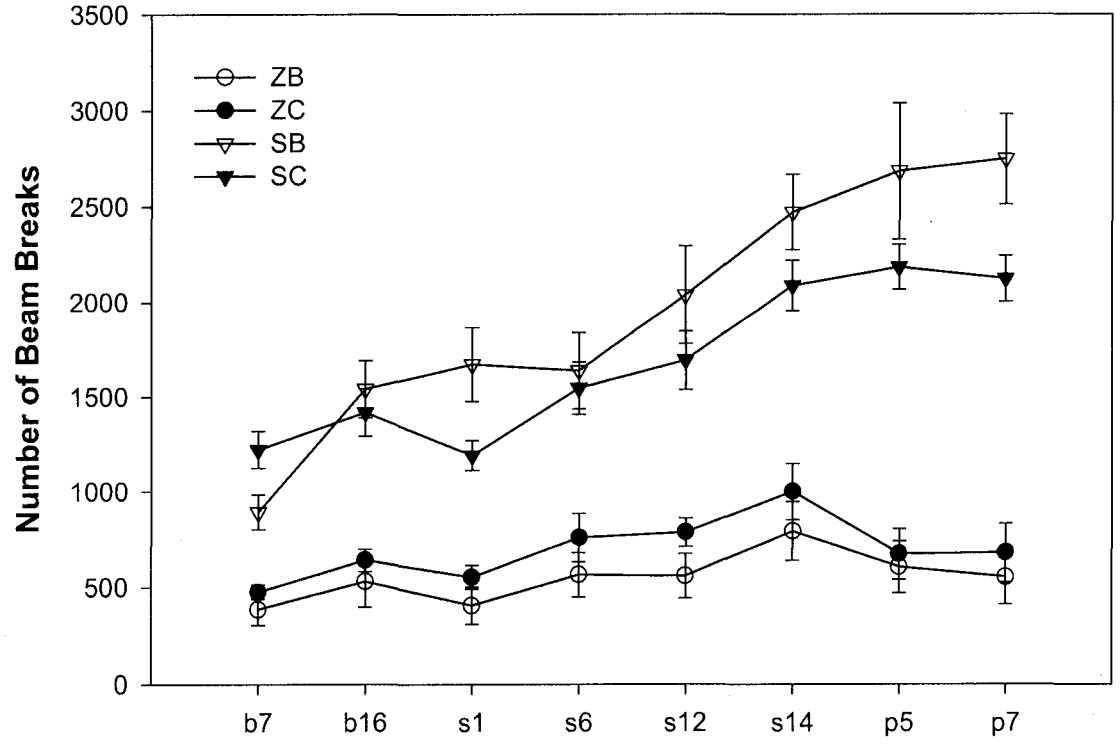

Figure 42. Vertical activity for each testing session.

EXPERIMENT II: FIGURES AND TABLES

Table 74. Experiment II Timeline.

Date	Day of Study	Phase	Day of Phase	Measure/Activity By Cohort (A or B)
9 Jan 07	Day 1			Rats arrive
10 Jan 07	Day 2	**Acclimation**	Day 1	ALL: Gentle, BW
11 Jan 07	Day 3		Day 2	ALL: Gentle, BW
12 Jan 07	Day 4		Day 3	ALL: Gentle, BW
15 Jan 07	Day 7		Day 6	A: FC, BW, OF B: FC, BW
16 Jan 07	Day 8		Day 7	B: OF
17 Jan 07	Day 9		Day 8	A: FC, BW B: FC, BW, ASR
18 Jan 07	Day 10		Day 9	B: ASR
19 Jan 07	Day 11	**Baseline**	Day 1	A: FC, BW, OF B: FC, BW
20 Jan 07	Day 12		Day 2	B: OF
21 Jan 07	Day 13		Day 3	A: FC, BW, ASR B: FC, BW
22 Jan 07	Day 14		Day 4	B: ASR
23 Jan 07	Day 15		Day 5	A: FC, BW, HP, HCA B: FC, BW, HCA
24 Jan 07	Day 16		Day 6	B: HP
25 Jan 07	Day 17		Day 7	A: FC, BW, ASR B: FC, BW
26 Jan 07	Day 18		Day 8	B: ASR
28 Jan 07	Day 20		Day 10	A: FC, BW B: FC, BW
29 Jan 07	Day 21	**Stress**	Day 1	A: OF B: Stress only
30 Jan 07	Day 22		Day 2	A: Stress only B: OF
31 Jan 07	Day 23		Day 3	A: FC, BW, ASR B: FC, BW
1 Feb 07	Day 24		Day 4	A: HCA B: ASR, HCA
2 Feb 07	Day 25		Day 5	A: FC, BW, EPM B: FC, BW
3 Feb 07	Day 26		Day 6	A: Stress only B: EPM
4 Feb 07	Day 27		Day 7	A: FC, BW B: FC, BW, HP
5 Feb 07	Day 28		Day 8	A: HP B: Stress only
6 Feb 07	Day 29		Day 9	A: FC, BW B: FC, BW, OF
7 Feb 07	Day 30		Day 10	A: OF B: Stress only
8 Feb 07	Day 31		Day 11	A: FC, BW, ASR B: FC, BW
9 Feb 07	Day 32		Day 12	A: HP B: ASR

Table 74. Experiment II Timeline(continued).

Date	Day of Study	Phase	Day of Phase	Measure/Activity By Cohort (A or B)
10 Feb 07	Day 33		Day 13	A: FC, BW B: FC, BW, HP
11 Feb 07	Day 34		Day 14	A and B: Stress only
12 Feb 07	Day 35	**Post-stress**	Day 1	A and B: FC, BW
13 Feb 07	Day 36		Day 2	A: HP
14 Feb 07	Day 37		Day 3	A: FC, BW, OF B: FC, BW, HP
15 Feb 07	Day 38		Day 4	A: ASR B: OF
16 Feb 07	Day 39		Day 5	A: FC, BW B: FC, BW
19 Feb 07	Day 42		Day 8	A: FC, BW, OF B: FC, BW
20 Feb 07	Day 43		Day 9	A: HP B: OF
21 Feb 07	Day 44		Day 10	A: FC, BW, HCA B: FC, BW, HP, HCA
22 Feb 07	Day 45		Day 11	A: ASR
23 Feb 07	Day 46		Day 12	A: FC, BW B: FC, BW, ASR
26 Feb 07	Day 49		Day 15	All: FC, BW, LI, Sacrifice

FC = food consumption
BW = body weight
LI = Lee index
OF = open field locomotor activity
ASR = acoustic startle response
HCA = home cage activity
HP = hot plate
A and B refer to cohorts of subjects for logistical purposes

Table 75. Descriptives for Body Weight.

Day	Baseline Day 10		Stress Day 9		Stress Day 11		Post-stress Day 2		Post-stress Day 4	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
LMC	220.2	23.3	276.1	27.3	282.7	27.8	299.4	29.6	301.8	30.6
LMS	219.2	15.4	269.9	14.0	277.5	12.2	293.7	11.7	293.4	11.5
OMC	276.2	19.9	369.6	24.6	384.7	24.4	411.6	24.0	417.2	23.1
OMS	269.5	20.2	360.8	27.3	372.2	26.5	399.9	28.8	406.2	29.5
LFC	151.1	2.5	177.3	6.3	179.1	7.1	187.9	7.8	190.1	6.9
LFS	149.3	4.1	172.1	2.9	173.6	6.5	183.7	7.8	186.4	4.8
OFC	288.4	20.6	361.3	24.7	374.0	23.0	395.7	26.4	397.4	23.3
OFS	287.7	18.1	357.1	22.0	367.3	19.2	386.8	21.6	392.8	21.8

Table 76. Descriptives for Lee Index.

Day	Post-stress Day 15		
Group	n	Mean	Std
LMC	8	5022.3	397.3
LMS	8	5018.2	257.0
OMC	7	7321.5	512.6
OMS	8	6999.2	507.3
LFC	8	3637.8	88.3
LFS	8	3437.3	125.6
OFC	8	6919.7	376.4
OFS	8	6880.7	398.7

Table 77. Descriptives for Corticosterone.

Day	Post-stress Day 15		
Group	n	Mean	Std
LMC	8	171.7	108.9
LMS	8	131.2	113.1
OMC	7	311.9	109.2
OMS	8	204.4	155.2
LFC	8	293.8	59.5
LFS	7	217.8	133.8
OFC	8	380.1	183.3
OFS	8	202.3	139.2

Table 78. Descriptives for Food Consumption.

Day	Baseline Day 10		Stress Day 9		Stress Day 11		Post-stress Day 2		Post-stress Day 4	
Group	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
LMC	51.4	5.5	53.1	5.1	42.9	4.2	39.3	4.2	73.2	7.1
LMS	48.9	2.5	51.2	5.3	43.3	4.1	37.0	3.1	71.5	4.9
OMC	75.4	6.6	78.8	13.7	65.7	4.5	57.4	4.6	102.0	7.9
OMS	73.2	8.3	69.5	7.1	65.9	6.5	55.6	6.5	101.4	10.3
LFC	38.8	2.7	42.2	3.4	29.3	1.4	28.7	2.0	53.3	1.9
LFS	39.3	2.2	42.9	4.5	29.8	3.8	30.6	2.3	54.1	4.8
OFC	69.7	4.9	65.6	3.9	58.0	8.3	49.4	3.4	93.1	7.4
OFS	74.1	6.3	65.2	6.2	56.6	5.6	50.9	5.3	91.7	8.3

Table 79. Descriptives for Home Cage Activity.

Day	Baseline Day 5		Stress Day 4		Post-stress Day 10	
Group	Mean	Std	Mean	Std	Mean	Std
LMC	2.6	0.3	2.3	0.4	2.1	0.4
LMS	2.7	0.2	2.4	0.5	3.4	0.4
OMC	1.4	0.3	2.0	0.3	1.6	0.5
OMS	1.4	0.3	2.0	0.3	1.6	0.5
LFC	1.4	0.3	2.1	0.5	1.7	0.4
LFS	2.8	0.5	2.4	0.4	2.3	0.5
OFC	2.7	0.4	3.0	0.4	2.7	0.4
OFS	2.0	0.2	2.2	0.4	1.9	0.4

Table 80. Descriptives for Open Field during Baseline Phase.

Day	Horizontal Activity		Vertical Activity		Center Time	
Group	Mean	Std	Mean	Std	Mean	Std
LMC	13836.5	3753.9	1141.3	567.0	659.0	309.3
LMS	12182.1	4215.3	782.3	428.4	367.6	137.3
OMC	7452.3	1996.0	211.3	254.8	319.1	100.3
OMS	8774.4	2377.5	501.4	262.5	439.0	228.4
LFC	16477.6	5307.9	1218.4	556.4	547.9	259.3
LFS	13371.8	3219.6	937.0	352.4	321.3	137.0
OFC	8634.6	1401.5	577.0	194.2	553.1	204.7
OFS	8707.1	1888.9	543.1	187.0	376.3	128.5

Table 81. Descriptives for Open Field during Stress Phase.

Day	Horizontal Activity		Vertical Activity		Center Time	
Group	Mean	Std	Mean	Std	Mean	Std
LMC	16586.8	2918.1	1897.3	435.0	1061.9	320.3
LMS	17454.3	3825.9	1727.9	570.3	766.1	380.8
OMC	8679.0	2269.0	385.0	534.0	1099.7	317.1
OMS	9009.6	2268.4	823.9	409.0	706.6	230.4
LFC	18672.8	5485.8	1867.9	793.4	907.5	577.0
LFS	17842.9	4490.5	1547.4	442.1	493.6	168.6
OFC	9771.5	1931.5	900.1	316.6	573.4	239.7
OFS	9773.5	1234.5	913.1	212.4	839.5	227.9

Table 82. Descriptives for Open Field during Post-stress Phase.

Day	Horizontal Activity		Vertical Activity		Center Time	
Group	Mean	Std	Mean	Std	Mean	Std
LMC	17472.4	4138.9	2169.3	591.6	1166.2	333.0
LMS	19957.3	2245.0	2406.9	306.9	1204.6	393.9
OMC	7905.3	1953.1	352.8	478.9	1288.3	507.9
OMS	7704.4	1153.6	782.1	342.3	679.3	322.4
LFC	20890.3	5160.3	2231.8	592.9	995.6	318.8
LFS	20182.1	1757.9	2246.9	320.2	574.2	143.9
OFC	9602.0	817.8	960.5	239.6	925.0	390.5
OFS	9857.0	1653.9	1089.3	267.1	869.1	400.6

Table 83. Descriptives for Startle Responses.

Group	Startle	Baseline Day 6			Stress Day 15			Post-stress Day 2		
		n	Mean	Std	n	Mean	Std	n	Mean	Std
LMC	110 dB	8	187.3	54.6	8	221.0	74.1	8	288.7	152.8
	120 dB	8	249.2	104.5	8	310.3	101.8	8	372.7	220.3
LMS	110 dB	8	257.0	140.6	8	286.8	112.4	8	418.6	179.6
	120 dB	8	343.6	153.3	8	368.2	103.1	8	568.4	305.0
OMC	110 dB	8	79.2	17.9	8	110.3	54.9	7	133.5	86.5
	120 dB	8	154.1	51.4	8	219.7	88.2	7	214.8	121.4
OMS	110 dB	8	129.2	60.2	8	126.7	64.4	8	85.7	51.2
	120 dB	8	164.9	88.9	8	199.5	89.8	8	211.9	141.4
LFC	110 dB	8	121.4	31.3	8	136.6	43.4	8	121.9	71.3
	120 dB	8	124.5	21.6	8	209.9	45.7	8	194.7	81.4
LFS	110 dB	8	106.6	38.2	8	82.8	47.9	8	102.9	23.6
	120 dB	8	116.5	45.1	8	118.6	46.6	8	139.5	44.2
OFC	110 dB	8	167.9	112.1	8	225.0	180.3	8	244.7	170.1
	120 dB	8	250.5	175.9	8	313.0	205.2	8	345.8	200.3
OFS	110 dB	8	130.5	51.6	8	153.8	60.7	8	189.5	94.7
	120 dB	8	186.6	86.5	8	218.2	66.4	8	310.1	104.4

Table 84. Descriptives for %PPI.

Group	Startle	Pre-pulse	Baseline Day 6			Stress Day 15			Post-stress Day 2		
			n	Mean	Std	n	Mean	Std	n	Mean	Std
LMC	110 dB	68 dB	8	16.8	30.8	8	29.7	15.9	8	43.0	40.1
	110 dB	82 dB	8	34.8	39.8	8	66.7	23.9	8	66.6	20.3
	120 dB	68 dB	8	10.1	11.5	8	19.7	29.6	8	41.2	24.5
	120 dB	82 dB	8	45.0	19.0	8	61.1	18.9	8	66.5	19.7
	Average %PPI		8	26.7	20.5	8	44.3	17.8	8	54.3	20.0
LMS	110 dB	68 dB	8	-0.1	33.9	8	21.0	40.3	8	33.1	33.5
	110 dB	82 dB	8	34.0	37.4	8	46.3	28.0	8	62.5	23.3
	120 dB	68 dB	8	10.0	22.2	8	29.0	20.9	8	20.4	34.3
	120 dB	82 dB	8	34.5	26.7	8	50.0	21.9	8	63.7	19.2
	Average %PPI		8	19.6	19.8	8	36.6	22.7	8	44.9	23.8
OMC	110 dB	68 dB	8	-14.7	47.5	8	27.9	26.9	7	29.6	23.7
	110 dB	82 dB	8	45.8	41.9	8	35.3	51.2	7	66.4	17.8
	120 dB	68 dB	8	11.9	41.8	8	12.2	23.3	7	14.8	17.3
	120 dB	82 dB	8	21.4	51.3	8	50.5	10.4	7	55.5	18.9
	Average %PPI		8	16.1	27.5	8	31.5	17.5	7	41.6	14.5
OMS	110 dB	68 dB	8	20.0	27.6	8	30.6	35.6	8	23.3	44.2
	110 dB	82 dB	8	38.6	15.3	8	56.4	25.0	8	33.7	65.6
	120 dB	68 dB	8	1.6	22.8	8	10.6	16.3	8	9.5	49.1
	120 dB	82 dB	8	15.7	21.9	8	55.2	25.6	8	55.3	17.0
	Average %PPI		8	19.0	15.9	8	38.2	11.5	8	30.4	24.7

Table 84. Descriptives for %PPI (continued).

Group	Startle	Pre-pulse	Baseline			Stress			Post-stress		
			Day 6			Day 15			Day 2		
			n	Mean	Std	n	Mean	Std	n	Mean	Std
LFC	110 dB	68 dB	8	14.5	38.5	8	13.7	31.3	8	14.7	40.6
	110 dB	82 dB	8	64.5	21.5	8	60.0	22.5	8	71.2	14.6
	120 dB	68 dB	8	-2.8	46.6	8	12.6	28.2	8	33.2	32.3
	120 dB	82 dB	8	47.1	25.3	8	68.6	12.2	8	69.4	23.2
	Average %PPI		8	30.8	25.1	8	38.7	11.4	8	47.1	18.2
LFS	110 dB	68 dB	8	31.7	19.0	8	33.7	36.2	8	64.4	19.2
	110 dB	82 dB	8	46.6	22.5	8	47.1	28.9	8	65.7	8.6
	120 dB	68 dB	8	10.7	27.0	8	13.7	67.5	8	31.3	37.1
	120 dB	82 dB	8	37.4	36.4	8	45.5	24.5	8	74.5	13.0
	Average %PPI		8	31.6	14.0	8	35.0	26.6	8	59.0	9.7
OFC	110 dB	68 dB	8	59.7	34.5	8	69.6	22.3	8	64.0	29.0
	110 dB	82 dB	8	77.4	10.4	8	69.5	22.3	8	88.1	10.7
	120 dB	68 dB	8	56.1	32.3	8	67.4	23.5	8	74.7	17.7
	120 dB	82 dB	8	67.5	22.1	8	74.7	24.2	8	84.0	16.1
	Average %PPI		8	65.2	21.2	8	70.3	17.5	8	77.7	13.5
OFS	110 dB	68 dB	8	11.1	25.5	8	0.4	24.9	8	1.6	34.7
	110 dB	82 dB	8	38.3	26.8	8	53.6	19.1	8	40.5	31.6
	120 dB	68 dB	8	1.3	45.2	8	14.8	27.1	8	-2.9	29.5
	120 dB	82 dB	8	19.9	30.8	8	42.8	20.9	8	39.2	34.5
	Average %PPI		8	17.7	20.9	8	27.9	19.0	8	19.6	17.1

Table 85. ANOVA for Mean Body Weight during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BODY TYPE	145933.550	1	145933.550	495.089	.000	0.898	1.000
SEX	11791.245	1	11791.245	40.003	.000	0.417	1.000
STRESS	103.276	1	103.276	.350	.556	0.006	.090
BODY TYPE X SEX	28726.013	1	28726.013	97.455	.000	0.635	1.000
BODY TYPE X STRESS	22.444	1	22.444	.076	.784	0.001	.058
SEX X STRESS	27.170	1	27.170	.092	.763	0.002	.060
BODY TYPE X SEX X STRESS	46.751	1	46.751	.159	.692	0.003	.068
Error	16506.674	56	294.762				

Table 86. ANCOVA for Mean Body Weight during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BODY WEIGHT	17135.495	1	17135.495	159.058	.000	0.743	1.000
BODY TYPE	3295.764	1	3295.764	30.592	.000	0.357	1.000
SEX	6453.499	1	6453.499	59.904	.000	0.521	1.000
STRESS	277.492	1	277.492	2.576	.114	0.045	.351
BODY TYPE X SEX	88.666	1	88.666	0.823	.368	0.015	.145
BODY TYPE X STRESS	0.078	1	0.078	0.001	.979	0.000	.050
SEX X STRESS	0.127	1	0.127	0.001	.973	0.000	.050
BODY TYPE X SEX X STRESS	4.164	1	4.164	0.039	.845	0.001	.054
Error	5925.212	55	107.731				

Table 87. ANCOVA for Mean Body Weight during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BODY WEIGHT	16586.070	1	16586.070	107.105	.000	0.661	1.000
BODY TYPE	6489.873	1	6489.873	41.908	.000	0.432	1.000
SEX	11840.698	1	11840.698	76.461	.000	0.582	1.000
STRESS	356.609	1	356.609	2.303	.135	0.040	.320
BODY TYPE X SEX	132.891	1	132.891	0.858	.358	0.015	.149
BODY TYPE X STRESS	5.380	1	5.380	0.035	.853	0.001	.054
SEX X STRESS	5.774	1	5.774	0.037	.848	0.001	.054
BODY TYPE X SEX X STRESS	28.370	1	28.370	0.183	.670	0.003	.071
Error	8517.219	55	154.859				

Table 88. Repeated-measures ANCOVA for Body Weight within Stress Phase.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	741.999	6	123.667	3.555	.002	0.061	.951
TIME X BASELINE BW	166.483	6	27.747	0.798	.572	0.014	.316
TIME X BODY TYPE	2304.772	6	384.129	11.042	.000	0.167	1.000
TIME X SEX	3085.185	6	514.198	14.781	.000	0.212	1.000
TIME X STRESS	432.033	6	72.005	2.070	.056	0.036	.746
TIME X BODY TYPE X SEX	160.699	6	26.783	0.770	.594	0.014	.305
TIME X BODY TYPE X STRESS	83.503	6	13.917	0.400	.879	0.007	.167
TIME X SEX X STRESS	13.602	6	2.267	0.065	.999	0.001	.066
TIME X BODY TYPE X SEX X STRESS	52.042	6	8.674	0.249	.959	0.005	.117
Error(TIME)	11479.609	330	34.787				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	116893.572	1	116893.572	354.321	.000	0.866	1.000
BODY TYPE	8941.995	1	8941.995	27.104	.000	0.330	.999
SEX	20418.323	1	20418.323	61.891	.000	0.529	1.000
STRESS	941.844	1	941.844	2.855	.097	0.049	.382
BODY TYPE X SEX	372.739	1	372.739	1.130	.292	0.020	.181
BODY TYPE X STRESS	3.251	1	3.251	0.010	.921	0.000	.051
SEX X STRESS	5.029	1	5.029	0.015	.902	0.000	.052
BODY TYPE X SEX X STRESS	0.986	1	0.986	0.003	.957	0.000	.050
ERROR	18144.983	55	329.909				

Table 89. Repeated-measures ANCOVA on Body Weight within Post-stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	445.150	6	74.192	2.655	.016	0.047	.860
TIME X BASELINE BW	102.132	6	17.022	0.609	.723	0.011	.243
TIME X BODY TYPE	1113.524	6	185.587	6.642	.000	0.110	.999
TIME X SEX	201.273	6	33.545	1.200	.306	0.022	.472
TIME X STRESS	2078.138	6	346.356	12.395	.000	0.187	1.000
TIME X BODY TYPE X SEX	171.666	6	28.611	1.024	.410	0.019	.405
TIME X BODY TYPE X STRESS	149.740	6	24.957	0.893	.500	0.016	.353
TIME X SEX X STRESS	182.049	6	30.341	1.086	.371	0.020	.429
TIME X BODY TYPE X SEX X STRESS	384.029	6	64.005	2.291	.035	0.041	.795
Error(TIME)	9053.558	324	27.943				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	128322.552	1	128322.552	123.765	.000	0.696	1.000
BODY TYPE	47549.795	1	47549.795	45.861	.000	0.459	1.000
SEX	3858.857	1	3858.857	3.722	.059	0.064	.474
STRESS	101046.236	1	101046.236	97.458	.000	0.643	1.000
BODY TYPE X SEX	704.406	1	704.406	0.679	.413	0.012	.128
BODY TYPE X STRESS	136.429	1	136.429	0.132	.718	0.002	.065
SEX X STRESS	747.226	1	747.226	0.721	.400	0.013	.133
BODY TYPE X SEX X STRESS	251.258	1	251.258	0.242	.625	0.004	.077
ERROR	55988.384	54	1036.822				

Table 90. Repeated-measures ANCOVA on Body Weight during Stress and Post-stress Phases.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	119.953	1	119.953	13.046	.001	0.192	.944
TIME X BASELINE BW	2.238	1	2.238	0.243	.624	0.004	.077
TIME X BODY TYPE	267.984	1	267.984	29.145	.000	0.346	1.000
TIME X SEX	405.592	1	405.592	44.111	.000	0.445	1.000
TIME X STRESS	2.478	1	2.478	0.269	.606	0.005	.080
TIME X BODY TYPE X SEX	2.229	1	2.229	0.242	.624	0.004	.077
TIME X BODY TYPE X STRESS	2.080	1	2.080	0.226	.636	0.004	.075
TIME X SEX X STRESS	2.093	1	2.093	0.228	.635	0.004	.076
TIME X BODY TYPE X SEX X STRESS	5.399	1	5.399	0.587	.447	0.011	.117
Error(TIME)	505.718	55	9.195				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE BW	33719.327	1	33719.327	133.070	.000	.708	1.000
BODY TYPE	9517.652	1	9517.652	37.561	.000	.406	1.000
SEX	17888.605	1	17888.605	70.596	.000	.562	1.000
STRESS	631.624	1	631.624	2.493	.120	.043	.342
BODY TYPE X SEX	219.328	1	219.328	0.866	.356	.015	.150
BODY TYPE X STRESS	3.378	1	3.378	0.013	.908	.000	.051
SEX X STRESS	3.809	1	3.809	0.015	.903	.000	.052
BODY TYPE X SEX X STRESS	27.136	1	27.136	0.107	.745	.002	.062
ERROR	13936.713	55	253.395				

Table 91. ANOVA on Food Consumption during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BODY TYPE	12991.440	1	12991.440	461.478	.000	0.892	1.000
SEX	738.481	1	738.481	26.232	.000	0.319	.999
STRESS	0.040	1	0.040	0.001	.970	0.000	.050
BODY TYPE X SEX	302.325	1	302.325	10.739	.002	0.161	.896
BODY TYPE X STRESS	18.169	1	18.169	0.645	.425	0.011	.124
SEX X STRESS	94.042	1	94.042	3.341	.073	0.056	.435
BODY TYPE X SEX X STRESS	12.638	1	12.638	0.449	.506	0.008	.101
Error	1576.500	56	28.152				

Table 92. ANCOVA for Food Consumption during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE FC	223.420	1	223.420	12.382	.001	0.184	.933
BODY TYPE	256.898	1	256.898	14.237	.000	0.206	.960
SEX	403.815	1	403.815	22.379	.000	0.289	.996
STRESS	74.739	1	74.739	4.142	.047	0.070	.516
BODY TYPE X SEX	0.144	1	0.144	0.008	.929	0.000	.051
BODY TYPE X STRESS	18.575	1	18.575	1.029	.315	0.018	.169
SEX X STRESS	50.384	1	50.384	2.792	.100	0.048	.375
BODY TYPE X SEX X STRESS	12.532	1	12.532	0.695	.408	0.012	.130
Error	992.429	55	18.044				

Table 93. ANCOVA for Food Consumption during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE FC	157.577	1	157.577	6.957	.011	0.112	.736
BODY TYPE	544.308	1	544.308	24.031	.000	0.304	.998
SEX	795.523	1	795.523	35.122	.000	0.390	1.000
STRESS	3.458	1	3.458	0.153	.698	0.003	.067
BODY TYPE X SEX	30.539	1	30.539	1.348	.251	0.024	.207
BODY TYPE X STRESS	3.102	1	3.102	0.137	.713	0.002	.065
SEX X STRESS	2.323	1	2.323	0.103	.750	0.002	.061
BODY TYPE X SEX X STRESS	10.895	1	10.895	0.481	.491	0.009	.105
Error	1245.753	55	22.650				

Table 94. Repeated-measures ANCOVA for Food Consumption within Stress Phase.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	590.977	5	118.195	3.770	.003	.064	.934
TIME X BASELINE FC	329.002	5	65.800	2.099	.066	.037	.692
TIME X BODY TYPE	260.743	5	52.149	1.663	.144	.029	.574
TIME X SEX	266.670	5	53.334	1.701	.134	.030	.585
TIME X STRESS	120.994	5	24.199	0.772	.571	.014	.276
TIME X BODY TYPE X SEX	56.717	5	11.343	0.362	.874	.007	.143
TIME X BODY TYPE X STRESS	133.401	5	26.680	0.851	.515	.015	.304
TIME X SEX X STRESS	178.685	5	35.737	1.140	.339	.020	.404
TIME X BODY TYPE X SEX X STRESS	77.198	5	15.440	0.492	.782	.009	.183
Error(TIME)	8621.730	275	31.352				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE FC	2974.459	1	2974.459	74.119	.000	0.574	1.000
BODY TYPE	643.716	1	643.716	16.040	.000	0.226	.976
SEX	1352.384	1	1352.384	33.699	.000	0.380	1.000
STRESS	165.822	1	165.822	4.132	.047	0.070	.515
BODY TYPE X SEX	0.929	1	0.929	0.023	.880	0.000	.053
BODY TYPE X STRESS	29.306	1	29.306	0.730	.397	0.013	.134
SEX X STRESS	51.254	1	51.254	1.277	.263	0.023	.199
BODY TYPE X SEX X STRESS	0.001	1	0.001	0.000	.996	0.000	.050
ERROR	2207.208	55	40.131				

Table 95. Repeated-measures ANCOVA for Food Consumption within Post-stress Phase.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	341.142	5	68.228	2.875	.015	.051	.841
TIME X BASELINE FC	90.452	5	18.090	0.762	.578	.014	.273
TIME X BODY TYPE	195.826	5	39.165	1.650	.147	.030	.570
TIME X SEX	56.394	5	11.279	0.475	.795	.009	.178
TIME X STRESS	224.985	5	44.997	1.896	.095	.034	.640
TIME X BODY TYPE X SEX	97.103	5	19.421	0.818	.537	.015	.292
TIME X BODY TYPE X STRESS	146.534	5	29.307	1.235	.293	.022	.436
TIME X SEX X STRESS	61.032	5	12.206	0.514	.765	.009	.190
TIME X BODY TYPE X SEX X STRESS	182.417	5	36.483	1.537	.178	.028	.535
Error(TIME)	6407.944	270	23.733				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE FC	1416.752	1	1416.752	14.819	.000	0.215	.966
BODY TYPE	2008.911	1	2008.911	21.013	.000	0.280	.994
SEX	120.214	1	120.214	1.257	.267	0.023	.196
STRESS	4180.447	1	4180.447	43.726	.000	0.447	1.000
BODY TYPE X SEX	131.319	1	131.319	1.374	.246	0.025	.210
BODY TYPE X STRESS	91.335	1	91.335	0.955	.333	0.017	.160
SEX X STRESS	3.290	1	3.290	0.034	.854	0.001	.054
BODY TYPE X SEX X STRESS	20.153	1	20.153	0.211	.648	0.004	.074
ERROR	5162.639	54	95.604				

Table 96. Repeated-measures ANCOVA on Food Consumption during Stress and Post-stress Phases.

Tests of Within-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	27.525	1	27.525	2.789	.101	.048	.375
TIME X BASELINE FC	2.866	1	2.866	0.290	.592	.005	.083
TIME X BODY TYPE	26.662	1	26.662	2.701	.106	.047	.365
TIME X SEX	23.023	1	23.023	2.333	.132	.041	.323
TIME X STRESS	32.885	1	32.885	3.332	.073	.057	.434
TIME X BODY TYPE X SEX	3.248	1	3.248	0.329	.569	.006	.087
TIME X BODY TYPE X STRESS	17.439	1	17.439	1.767	.189	.031	.257
TIME X SEX X STRESS	15.534	1	15.534	1.574	.215	.028	.234
TIME X BODY TYPE X SEX X STRESS	23.398	1	23.398	2.371	.129	.041	.328
Error(TIME)	542.877	55	9.870				
Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE FC	378.131	1	378.131	12.268	.001	.182	.931
BODY TYPE	774.544	1	774.544	25.128	.000	.314	.998
SEX	55.174	1	55.174	1.790	.186	.032	.260
STRESS	1166.453	1	1166.453	37.843	.000	.408	1.000
BODY TYPE X SEX	18.429	1	18.429	0.598	.443	.011	.118
BODY TYPE X STRESS	13.244	1	13.244	0.430	.515	.008	.099
SEX X STRESS	37.173	1	37.173	1.206	.277	.021	.190
BODY TYPE X SEX X STRESS	0.029	1	0.029	0.001	.976	.000	.050
ERROR	1695.305	55	30.824				

Table 97. ANOVA on Home Cage Activity during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BODY TYPE	16.759	1	16.759	134.823	.000	0.707	1.000
SEX	1.806	1	1.806	14.526	.000	0.206	0.963
STRESS	0.001	1	0.001	0.008	.930	0.000	0.051
BODY TYPE X SEX	0.712	1	0.712	5.727	.020	0.093	0.652
BODY TYPE X STRESS	0.009	1	0.009	0.071	.791	0.001	0.058
SEX X STRESS	0.048	1	0.048	0.385	.537	0.007	0.094
BODY TYPE X SEX X STRESS	0.165	1	0.165	1.328	.254	0.023	0.205
Error	6.961	56	0.124				

Table 98. ANCOVA on HCA during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE HCA	0.031	1	0.031	0.196	.660	0.004	0.072
BODY TYPE	1.655	1	1.655	10.621	.002	0.162	0.893
SEX	0.000	1	0.000	0.001	.973	0.000	0.050
STRESS	2.925	1	2.925	18.772	.000	0.254	0.989
BODY TYPE X SEX	1.597	1	1.597	10.248	.002	0.157	0.882
BODY TYPE X STRESS	0.041	1	0.041	0.262	.611	0.005	0.079
SEX X STRESS	1.804	1	1.804	11.579	.001	0.174	0.917
BODY TYPE X SEX X STRESS	0.032	1	0.032	0.206	.651	0.004	0.073
Error	8.570	55	0.156				

Table 99. ANCOVA on HCA during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE HCA	0.256	1	0.256	1.487	.228	0.026	0.224
BODY TYPE	2.212	1	2.212	12.827	.001	0.189	0.940
SEX	0.098	1	0.098	0.569	.454	0.010	0.115
STRESS	3.656	1	3.656	21.199	.000	0.278	0.995
BODY TYPE X SEX	0.503	1	0.503	2.914	.093	0.050	0.389
BODY TYPE X STRESS	2.208	1	2.208	12.800	.001	0.189	0.940
SEX X STRESS	0.543	1	0.543	3.147	.082	0.054	0.414
BODY TYPE X SEX X STRESS	0.775	1	0.775	4.494	.039	0.076	0.549
Error	9.486	55	0.172				

Table 100. Chi-Square for Home Cage Behaviors during Baseline Phase

Dependent Variable and (df)	Condition	N	Mean Rank
Eating Body Type: χ^2 (1) = 1.854, p = ns Stress: χ^2 (1) = 0.866, p = ns Sex: χ^2 (1) = 0.314, p = ns	Lean	16	18.63
	Obese	16	14.38
	Control	16	15.03
	Stress	16	17.97
	Males	16	17.38
	Females	16	15.63
Grooming Body Type: χ^2 (1) = 0.190, p = ns Stress: χ^2 (1) = 0.100, p = ns Sex: χ^2 (1) = 0.692, p = ns	Lean	16	17.19
	Obese	16	15.81
	Control	16	16.00
	Stress	16	17.00
	Males	16	17.81
	Females	16	15.19
Awake/not moving Body Type: χ^2 (1) = 13.220, p < 0.05 Stress: χ^2 (1) = 0.128, p = ns Sex: χ^2 (1) = 0.600, p = ns	Lean	16	10.78
	Obese	16	22.22
	Control	16	15.94
	Stress	16	17.06
	Males	16	15.28
	Females	16	17.72
Horizontal Activity Body Type: χ^2 (1) = 0.070, p = ns Stress: χ^2 (1) = 0.863, p = ns Sex: χ^2 (1) = 0.440, p = ns	Lean	16	16.13
	Obese	16	16.88
	Control	16	15.19
	Stress	16	17.81
	Males	16	17.44
	Females	16	15.56
Vertical Activity Body Type: χ^2 (1) = 20.390, p < 0.05 Stress: χ^2 (1) = 0.896, p = ns Sex: χ^2 (1) = 0.006, p = ns	Lean	16	23.66
	Obese	16	9.34
	Control	16	15.00
	Stress	16	18.00
	Males	16	16.63
	Females	16	16.38
Sleeping Body Type: χ^2 (1) = 11.201, p < 0.05 Stress: χ^2 (1) = 7.654, p = p < 0.05 Sex: χ^2 (1) = 0.337, p = ns	Lean	16	11.81
	Obese	16	21.19
	Control	16	20.38
	Stress	16	12.63
	Males	16	17.31
	Females	16	15.69

Table 101. Chi-Square for Home Cage Behaviors during Stress Phase.

Dependent Variable and (df)	Condition	N	Mean Rank
Eating Body Type: χ^2 (1) = 4.548, p < 0.05 Stress: χ^2 (1) = 0.014, p = ns Sex: χ^2 (1) = 2.003, p = ns	Lean	16	13.16
	Obese	16	19.84
	Control	16	16.31
	Stress	16	16.69
	Males	16	14.28
	Females	16	18.72
Grooming Body Type: χ^2 (1) = 4.056, p < 0.05 Stress: χ^2 (1) = 3.019, p = 0.082 Sex: χ^2 (1) = 0.126, p = ns	Lean	16	19.69
	Obese	16	13.31
	Control	16	13.75
	Stress	16	19.25
	Males	16	17.06
	Females	16	15.94
Awake/not moving Body Type: χ^2 (1) = 5.252, p < 0.05 Stress: χ^2 (1) = 0.003, p = ns Sex: χ^2 (1) = 0.240, p = ns	Lean	16	12.84
	Obese	16	20.16
	Control	16	16.59
	Stress	16	16.41
	Males	16	15.72
	Females	16	17.28
Horizontal Activity Body Type: χ^2 (1) = 3.015, p < 0.05 Stress: χ^2 (1) = 0.788, p = ns Sex: χ^2 (1) = 1.265, p = ns	Lean	16	19.25
	Obese	16	13.75
	Control	16	17.91
	Stress	16	15.09
	Males	16	18.28
	Females	16	14.72
Vertical Activity Body Type: χ^2 (1) = 4.134, p < 0.05 Stress: χ^2 (1) = 0.813, p = ns Sex: χ^2 (1) = 3.114, p = 0.078	Lean	16	19.81
	Obese	16	13.19
	Control	16	17.97
	Stress	16	15.03
	Males	16	13.63
	Females	16	19.38
Sleeping Body Type: χ^2 (1) = 5.169, p < 0.05 Stress: χ^2 (1) = 0.141, p = ns Sex: χ^2 (1) = 4.438, p < 0.05	Lean	16	13.09
	Obese	16	19.91
	Control	16	15.94
	Stress	16	17.06
	Males	16	19.66
	Females	16	13.34

Table 102. Chi-Square for Home Cage Behaviors during Post-stress Phase.

Dependent Variable and (df)	Condition	N	Mean Rank
Eating Body Type: χ^2 (1) = 7.302, p = ns Stress: χ^2 (1) = 0.058, p = ns Sex: χ^2 (1) = 0.361, p = ns	Lean	16	12.28
	Obese	16	20.72
	Control	16	16.88
	Stress	16	16.13
	Males	16	15.56
	Females	16	17.44
Grooming Body Type: χ^2 (1) = 0.460, p = ns Stress: χ^2 (1) = 7.468, p < 0.05 Sex: χ^2 (1) = 0.014, p = ns	Lean	16	17.59
	Obese	16	15.41
	Control	16	12.09
	Stress	16	20.91
	Males	16	16.69
	Females	16	16.31
Awake/not moving Body Type: χ^2 (1) = 7.262, p = ns Stress: χ^2 (1) = 1.367, p = ns Sex: χ^2 (1) = 2.389, p = ns	Lean	16	12.25
	Obese	16	20.75
	Control	16	18.34
	Stress	16	14.66
	Males	16	18.94
	Females	16	14.06
Horizontal Activity Body Type: χ^2 (1) = 0.027, p = ns Stress: χ^2 (1) = 1.228, p = ns Sex: χ^2 (1) = 0.095, p = ns	Lean	16	16.25
	Obese	16	16.75
	Control	16	18.19
	Stress	16	14.81
	Males	16	16.03
	Females	16	16.97
Vertical Activity Body Type: χ^2 (1) = 14.740, p = ns Stress: χ^2 (1) = 0.329, p = ns Sex: χ^2 (1) = 3.995, p < 0.05	Lean	16	22.56
	Obese	16	10.44
	Control	16	17.41
	Stress	16	15.59
	Males	16	13.34
	Females	16	19.66
Sleeping Body Type: χ^2 (1) = 4.345, p = ns Stress: χ^2 (1) = 0.000, p = ns Sex: χ^2 (1) = 1.871, p = ns	Lean	16	13.50
	Obese	16	19.50
	Control	16	16.53
	Stress	16	16.47
	Males	16	18.47
	Females	16	14.53

Table 103. ANOVA for Horizontal Activity during Baseline Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BODY TYPE	497273275.141	1	497273275.141	46.491	.000	.454	1.000
SEX	24461679.516	1	24461679.516	2.287	.136	.039	.318
STRESS	11327431.641	1	11327431.641	1.059	.308	.019	.173
BODY TYPE X SEX	7374619.141	1	7374619.141	.689	.410	.012	.129
SEX X STRESS	7296076.266	1	7296076.266	.682	.412	.012	.128
BODY TYPE X STRESS	37882486.266	1	37882486.266	3.542	.065	.059	.456
BODY TYPE X SEX X STRESS	40753.516	1	40753.516	.004	.951	.000	.050
Error	598978226.375	56	10696039.757				

Table 104. ANOVA for Vertical Activity during Baseline Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BODY TYPE	5045077.516	1	5045077.516	35.242	.000	.386	1.000
SEX	408800.391	1	408800.391	2.856	.097	.049	.383
STRESS	147552.016	1	147552.016	1.031	.314	.018	.170
BODY TYPE X SEX	30844.141	1	30844.141	.215	.644	.004	.074
SEX X STRESS	60700.641	1	60700.641	.424	.518	.008	.098
BODY TYPE X STRESS	803936.391	1	803936.391	5.616	.021	.091	.644
BODY TYPE X SEX X STRESS	161302.641	1	161302.641	1.127	.293	.020	.181
Error	8016653.125	56	143154.520				

Table 105. ANCOVA on Horizontal Activity during Stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE HA	90218792.535	1	90218792.535	9.318	.003	.145	.851
BODY TYPE	332496689.886	1	332496689.886	34.342	.000	.384	1.000
SEX	5587199.042	1	5587199.042	.577	.451	.010	.116
STRESS	2758311.707	1	2758311.707	.285	.596	.005	.082
BODY TYPE X SEX	187510.586	1	187510.586	.019	.890	.000	.052
SEX X STRESS	944384.033	1	944384.033	.098	.756	.002	.061
BODY TYPE X STRESS	4122843.851	1	4122843.851	.426	.517	.008	.098
BODY TYPE X SEX X STRESS	1665025.148	1	1665025.148	.172	.680	.003	.069
Error	532505636.715	55	9681920.668				

Table 106. ANCOVA on Vertical Activity during Stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE VA	1309134.169	1	1309134.169	5.877	.019	.097	.663
BODY TYPE	5938465.801	1	5938465.801	26.660	.000	.326	.999
SEX	17630.838	1	17630.838	.079	.780	.001	.059
STRESS	13493.876	1	13493.876	.061	.806	.001	.057
BODY TYPE X SEX	550345.981	1	550345.981	2.471	.122	.043	.339
SEX X STRESS	226234.354	1	226234.354	1.016	.318	.018	.168
BODY TYPE X STRESS	305126.438	1	305126.438	1.370	.247	.024	.210
BODY TYPE X SEX X STRESS	12395.777	1	12395.777	.056	.814	.001	.056
Error	12251017.581	55	222745.774				

Table 107. ANCOVA on Horizontal Activity during Post-stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE HA	183719456.224	1	183719456.224	42.216	.000	.434	1.000
BODY TYPE	527904125.902	1	527904125.902	121.305	.000	.688	1.000
SEX	21704421.473	1	21704421.473	4.987	.030	.083	.593
STRESS	13398461.800	1	13398461.800	3.079	.085	.053	.407
BODY TYPE X SEX	2890578.242	1	2890578.242	.664	.419	.012	.126
SEX X STRESS	1521979.236	1	1521979.236	.350	.557	.006	.089
BODY TYPE X STRESS	24764465.688	1	24764465.688	5.691	.021	.094	.649
BODY TYPE X SEX X STRESS	12510025.094	1	12510025.094	2.875	.096	.050	.384
Error	239353686.901	55	4351885.216				

Table 108. ANCOVA on Vertical Activity during Post-stress Phase.

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE VA	2694508.772	1	2694508.772	21.417	.000	.280	.995
BODY TYPE	12806504.065	1	12806504.065	101.790	.000	.649	1.000
SEX	189855.158	1	189855.158	1.509	.225	.027	.227
STRESS	1048965.693	1	1048965.693	8.337	.006	.132	.810
BODY TYPE X SEX	825934.114	1	825934.114	6.565	.013	.107	.711
SEX X STRESS	143532.369	1	143532.369	1.141	.290	.020	.183
BODY TYPE X STRESS	41795.808	1	41795.808	.332	.567	.006	.087
BODY TYPE X SEX X STRESS	23465.642	1	23465.642	.187	.668	.003	.071
Error	6919739.853	55	125813.452				

Table 109. ANOVA for 110 dB startle during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BODY TYPE	27440.958	1	27440.958	4.946	.030	.081	.589
SEX	15976.170	1	15976.170	2.880	.095	.049	.385
STRESS	4538.970	1	4538.970	.818	.370	.014	.144
BODY TYPE X SEX	93823.519	1	93823.519	16.913	.000	.232	.981
BODY TYPE X STRESS	1787.704	1	1787.704	.322	.573	.006	.086
SEX X STRESS	29556.057	1	29556.057	5.328	.025	.087	.621
BODY TYPE X SEX X STRESS	8.248	1	8.248	.001	.969	.000	.050
Error	310663.360	56	5547.560				

Table 110. ANOVA on 120 dB startle during Baseline Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BODY TYPE	6017.881	1	6017.881	.560	.457	.010	.114
SEX	54615.690	1	54615.690	5.086	.028	.083	.601
STRESS	1100.581	1	1100.581	.102	.750	.002	.061
BODY TYPE X SEX	220712.040	1	220712.040	20.555	.000	.269	.994
BODY TYPE X STRESS	19425.391	1	19425.391	1.809	.184	.031	.262
SEX X STRESS	31399.840	1	31399.840	2.924	.093	.050	.390
BODY TYPE X SEX X STRESS	767.290	1	767.290	.071	.790	.001	.058
Error	601303.593	56	10737.564				

Table 111. ANCOVA on 110 dB during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE 110 dB	154774.566	1	154774.566	27.917	.000	.337	.999
BODY TYPE	28.422	1	28.422	.005	.943	.000	.051
SEX	3149.664	1	3149.664	.568	.454	.010	.115
STRESS	8043.987	1	8043.987	1.451	.234	.026	.220
BODY TYPE X SEX	35201.261	1	35201.261	6.349	.015	.103	.697
BODY TYPE X STRESS	1353.365	1	1353.365	.244	.623	.004	.077
SEX X STRESS	6720.665	1	6720.665	1.212	.276	.022	.191
BODY TYPE X SEX X STRESS	1158.662	1	1158.662	.209	.649	.004	.073
Error	304930.128	55	5544.184				

Table 112. ANCOVA on 120 dB startle during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE 120 dB	282538.258	1	282538.258	46.987	.000	.461	1.000
BODY TYPE	11.440	1	11.440	.002	.965	.000	.050
SEX	5538.786	1	5538.786	.921	.341	.016	.156
STRESS	29227.067	1	29227.067	4.861	.032	.081	.582
BODY TYPE X SEX	14314.605	1	14314.605	2.381	.129	.041	.329
BODY TYPE X STRESS	190.222	1	190.222	.032	.859	.001	.054
SEX X STRESS	9937.908	1	9937.908	1.653	.204	.029	.244
BODY TYPE X SEX X STRESS	3097.127	1	3097.127	.515	.476	.009	.109
Error	330723.822	55	6013.160				

Table 113. ANCOVA on %PPI during Stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE AVERAGE %PPI	1882.647	1	1882.647	5.906	.018	.097	.666
BODY TYPE	115.764	1	115.764	.363	.549	.007	.091
SEX	11.946	1	11.946	.037	.847	.001	.054
STRESS	991.845	1	991.845	3.111	.083	.054	.410
BODY TYPE X SEX	700.918	1	700.918	2.199	.144	.038	.308
BODY TYPE X STRESS	175.990	1	175.990	.552	.461	.010	.113
SEX X STRESS	1038.689	1	1038.689	3.258	.077	.056	.426
BODY TYPE X SEX X STRESS	1206.585	1	1206.585	3.785	.057	.064	.481
Error	17533.388	55	318.789				

Table 114. ANCOVA on 110 dB startle during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE 110 dB	192786.419	1	192786.419	18.443	.000	.255	.988
BODY TYPE	20532.713	1	20532.713	1.964	.167	.035	.280
SEX	25492.908	1	25492.908	2.439	.124	.043	.335
STRESS	1771.677	1	1771.677	.169	.682	.003	.069
BODY TYPE X SEX	159850.544	1	159850.544	15.292	.000	.221	.970
BODY TYPE X STRESS	30928.988	1	30928.988	2.959	.091	.052	.394
SEX X STRESS	490.327	1	490.327	.047	.829	.001	.055
BODY TYPE X SEX X STRESS	19590.649	1	19590.649	1.874	.177	.034	.270
Error	564475.200	54	10453.244				

Table 115. ANCOVA on 120 dB startle during Post-stress Phase.

Tests of Between-Subjects Effects							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE 120 dB	581692.386	1	581692.386	29.778	.000	.355	1.000
BODY TYPE	13648.967	1	13648.967	.699	.407	.013	.130
SEX	19122.140	1	19122.140	.979	.327	.018	.163
STRESS	5010.251	1	5010.251	.256	.615	.005	.079
BODY TYPE X SEX	102347.142	1	102347.142	5.239	.026	.088	.613
BODY TYPE X STRESS	1520.838	1	1520.838	.078	.781	.001	.059
SEX X STRESS	11606.766	1	11606.766	.594	.444	.011	.118
BODY TYPE X SEX X STRESS	35004.410	1	35004.410	1.792	.186	.032	.260
Error	1054841.604	54	19534.104				

Table 116. ANCOVA on %PPI during Post-stress Phase.

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE AVERAGE %PPI	3277.617	1	3277.617	11.550	.001	.176	.916
BODY TYPE	1640.886	1	1640.886	5.782	.020	.097	.656
SEX	83.798	1	83.798	.295	.589	.005	.083
STRESS	1864.643	1	1864.643	6.571	.013	.108	.711
BODY TYPE X SEX	65.861	1	65.861	.232	.632	.004	.076
BODY TYPE X STRESS	2849.760	1	2849.760	10.042	.003	.157	.875
SEX X STRESS	120.641	1	120.641	.425	.517	.008	.098
BODY TYPE X SEX X STRESS	2025.543	1	2025.543	7.138	.010	.117	.747
Error	15324.493	54	283.787				

Table 117. Repeated-measures ANVOA on 110 dB startle.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	1919.825	1	1919.825	.394	.533	.007	.095
TIME * BASELINE 110 dB	1032.312	1	1032.312	.212	.647	.004	.074
TIME * BODY TYPE	10807.545	1	10807.545	2.215	.142	.039	.310
TIME * SEX	5614.333	1	5614.333	1.151	.288	.021	.184
TIME * STRESS	1019.418	1	1019.418	.209	.649	.004	.073
TIME * BODY TYPE * SEX	22420.463	1	22420.463	4.595	.037	.078	.558
TIME * BODY TYPE * STRESS	9976.545	1	9976.545	2.045	.158	.036	.290
TIME * SEX * STRESS	1851.556	1	1851.556	.380	.540	.007	.093
TIME * BODY TYPE * SEX * STRESS	5834.743	1	5834.743	1.196	.279	.022	.189
Error(time)	263454.819	54	4878.793				

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE 110 dB	346703.696	1	346703.696	30.906	.000	.364	1.000
BODY TYPE	9739.067	1	9739.067	.868	.356	.016	.150
SEX	22762.221	1	22762.221	2.029	.160	.036	.288
STRESS	8363.909	1	8363.909	.746	.392	.014	.136
BODY TYPE X SEX	172795.281	1	172795.281	15.404	.000	.222	.971
BODY TYPE X STRESS	22150.364	1	22150.364	1.975	.166	.035	.281
SEX X STRESS	5527.193	1	5527.193	.493	.486	.009	.106
BODY TYPE X SEX X STRESS	14776.147	1	14776.147	1.317	.256	.024	.204
Error	605765.874	54	11217.887				

Table 118. Repeated-measures ANCOVA on 120 dB startle.

Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	707.483	1	707.483	.080	.778	.001	.059
TIME * BASELINE 120 dB	27019.112	1	27019.112	3.062	.086	.054	.405
TIME * BODY TYPE	7337.950	1	7337.950	.832	.366	.015	.146
TIME * SEX	1606.400	1	1606.400	.182	.671	.003	.070
TIME * STRESS	30758.093	1	30758.093	3.485	.067	.061	.450
TIME * BODY TYPE * SEX	21425.730	1	21425.730	2.428	.125	.043	.334
TIME * BODY TYPE * STRESS	1001.501	1	1001.501	.113	.738	.002	.063
TIME * SEX * STRESS	131.112	1	131.112	.015	.903	.000	.052
TIME * BODY TYPE * SEX * STRESS	7697.517	1	7697.517	.872	.354	.016	.151
Error(time)	476530.278	54	8824.635				

Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE 120 dB	835813.281	1	835813.281	49.842	.000	.480	1.000
BODY TYPE	6329.639	1	6329.639	.377	.542	.007	.093
SEX	24174.506	1	24174.506	1.442	.235	.026	.218
STRESS	5666.687	1	5666.687	.338	.563	.006	.088
BODY TYPE X SEX	93670.410	1	93670.410	5.586	.022	.094	.641
BODY TYPE X STRESS	552.480	1	552.480	.033	.857	.001	.054
SEX X STRESS	19855.479	1	19855.479	1.184	.281	.021	.188
BODY TYPE X SEX X STRESS	31278.153	1	31278.153	1.865	.178	.033	.269
Error	905547.084	54	16769.390				

Table 119. Repeated-measures ANCOVA on %PPI.

Tests of Within-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
TIME	122.557	1	122.557	.549	.462	.010	.113
TIME * BASELINE%PPI	113.434	1	113.434	.508	.479	.009	.108
TIME * BODY TYPE	1318.635	1	1318.635	5.910	.018	.099	.666
TIME * SEX	16.635	1	16.635	.075	.786	.001	.058
TIME * STRESS	72.015	1	72.015	.323	.572	.006	.086
TIME * BODY TYPE * SEX	164.130	1	164.130	.736	.395	.013	.134
TIME * BODY TYPE * STRESS	803.324	1	803.324	3.601	.063	.063	.462
TIME * SEX * STRESS	221.392	1	221.392	.992	.324	.018	.165
TIME * BODY TYPE * SEX * STRESS	54.082	1	54.082	.242	.624	.004	.077
Error(time)	12047.768	54	223.107				

Tests of Between-Subjects Effects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
BASELINE AVERAGE %PPI	4944.038	1	4944.038	12.830	.001	.192	.940
BODY TYPE	439.898	1	439.898	1.142	.290	.021	.183
SEX	78.629	1	78.629	.204	.653	.004	.073
STRESS	2764.839	1	2764.839	7.175	.010	.117	.749
BODY TYPE X SEX	589.922	1	589.922	1.531	.221	.028	.229
BODY TYPE X STRESS	2223.329	1	2223.329	5.770	.020	.097	.655
SEX X STRESS	924.922	1	924.922	2.400	.127	.043	.331
BODY TYPE X SEX X STRESS	3169.027	1	3169.027	8.224	.006	.132	.804
Error	20808.187	54	385.337				

Table 120. Paired t-test Comparing Changes between Phases for Each Group.

Group	Baseline Phase to Stress Phase Comparison	t value (df)	p value	Stress Phase to Post-stress Phase Comparison	t value (df)	p value
LMC	BW	-24.962 (7)	.000	BW	-17.070 (7)	.000
	bland food	2.357 (7)	.051	bland food	-7.411 (7)	.000
	HCA	1.247 (7)	.252	HCA	1.507 (7)	.175
	HZ	-2.117 (7)	.072	HZ	-0.656 (7)	.533
	VA	-3.818 (7)	.007	VA	-1.594 (7)	.155
	CD	-5.562 (7)	.001	CD	-1.805 (7)	.114
	CT	-4.585 (7)	.003	CT	-1.437 (7)	.194
	110 dB	-1.572 (7)	.160	110 dB	-1.572 (7)	.160
	120 dB	-2.570 (7)	.037	120 dB	-1.206 (7)	.267
	Average %PPI	-2.441 (7)	.045	Average %PPI	-1.388 (7)	.208
	HP	0.199 (7)	.848	HP	2.224 (7)	.062
LMS	BW	-23.882 (7)	.000	BW	-8.461 (7)	.000
	bland food	2.391 (7)	.048	bland food	-6.139 (7)	.000
	HCA	2.183 (7)	.065	HCA	-5.814 (7)	.001
	HZ	-3.073 (7)	.018	HZ	-1.972 (7)	.089
	VA	-3.524 (7)	.010	VA	-2.419 (7)	.046
	CD	-4.767 (7)	.002	CD	-2.756 (7)	.028
	CT	-2.941 (7)	.022	CT	-3.323 (7)	.013
	110 dB	-0.586 (7)	.576	110 dB	-2.114 (7)	.072
	120 dB	-0.467 (7)	.655	120 dB	-1.883 (7)	.102
	Average %PPI	-2.210 (7)	.063	Average %PPI	-0.826 (7)	.463
OMC	BW	-20.387 (7)	.000	BW	-22.970(7)	.000
	bland food	0.757 (7)	.474	bland food	-2.454 (7)	.044
	HCA	-3.211 (7)	.015	HCA	2.201 (7)	.064
	HZ	-1.545 (7)	.166	HZ	0.915 (7)	.391
	VA	-0.770 (7)	.466	VA	0.109 (7)	.917
	CD	-3.273 (7)	.014	CD	0.0766 (7)	.941
	CT	-6.505 (7)	.000	CT	-0.955 (7)	.371
	110 dB	0.261 (7)	.801	110 dB	-0.104 (6)	.920
	120 dB	-2.101 (7)	.074	120 dB	-1.555 (6)	.171
	Average %PPI	-1.840 (7)	.108	Average %PPI	-1.699 (6)	.140
	HP	-1.750 (7)	.124	HP	-0.503 (7)	.633

Table 120 (continued). Paired t-test Comparing Changes between Phases for Each Group.

Group	Baseline Phase to Stress Phase Comparison	t value (df)	p value	Stress Phase to Post-stress Phase Comparison	t value (df)	p value
OMS	BW	-13.268 (7)	.000	BW	-18.097 (7)	.000
	bland food	2.351 (7)	.051	bland food	-7.157 (7)	.000
	HCA	-4.245 (7)	.004	HCA	1.879 (7)	.102
	HZ	-0.198 (7)	.848	HZ	1.710 (7)	.131
	VA	-1.870 (7)	.104	VA	0.235 (7)	.821
	CD	-0.788 (7)	.456	CD	0.738 (7)	.484
	CT	-5.480 (7)	.001	CT	0.218 (7)	.834
	110 dB	0.825 (7)	.436	110 dB	1.340 (7)	.222
	120 dB	0.096 (7)	.926	120 dB	1.270 (7)	.245
	Average %PPI	-2.533 (7)	.039	Average %PPI	0.883 (7)	.407
	HP	-3.314 (7)	.013	HP	-0.011 (7)	.992
LFC	BW	-13.808 (7)	.000	BW	-14.430 (7)	.000
	bland food	1.846 (7)	.107	bland food	-4.207 (7)	.004
	HCA	2.311 (7)	.054	HCA	0.513 (7)	.623
	HZ	-1.581 (7)	.158	HZ	-2.344 (7)	.052
	VA	-3.678 (7)	.008	VA	-2.103 (7)	.074
	CD	-4.120 (7)	.004	CD	-2.140 (7)	.070
	CT	-2.299 (7)	.055	CT	-0.510 (7)	.626
	110 dB	1.678 (7)	.137	110 dB	3.698 (7)	.008
	120 dB	-0.964 (7)	.367	120 dB	0.688 (7)	.513
	Average %PPI	-0.922 (7)	.387	Average %PPI	-1.358 (7)	.217
	HP	-2.555 (7)	.038	HP	-0.100 (7)	.923
LFS	BW	-12.397 (7)	.000	BW	-11.423 (7)	.000
	bland food	1.089 (7)	.312	bland food	-6.555 (7)	.000
	HCA	-1.557 (7)	.164	HCA	2.582 (7)	.036
	HZ	-2.356 (7)	.051	HZ	-1.306 (7)	.233
	VA	-3.717 (7)	.007	VA	-3.320 (7)	.013
	CD	-3.121 (7)	.017	CD	-0.457 (7)	.661
	CT	-2.085 (7)	.076	CT	-0.838 (7)	.430
	110 dB	3.975 (7)	.005	110 dB	2.427 (7)	.046
	120 dB	1.736 (7)	.126	120 dB	-1.451 (7)	.190
	Average %PPI	-0.313 (7)	.764	Average %PPI	-2.670 (7)	.032
	HP	-0.579 (7)	.581	HP	-2.907 (7)	.023

Table 120 (continued). Paired t-test Comparing Changes between Phases for Each Group.

Group	Baseline Phase to Stress Phase Comparison	t value (df)	p value	Stress Phase to Post-stress Phase Comparison	t value (df)	p value
OFC	BW	-31.120 (7)	.000	BW	-25.770 (7)	.000
	bland food	6.000 (7)	.001	bland food	-6.398 (7)	.000
	HCA	3.756 (7)	.007	HCA	-4.545 (7)	.003
	HZ	-1.680 (7)	.137	HZ	0.263 (7)	.800
	VA	-3.298 (7)	.013	VA	-0.400 (7)	.701
	CD	-1.866 (7)	.104	CD	-1.519 (7)	.173
	CT	-0.212 (7)	.838	CT	-1.976 (7)	.089
	110 dB	2.037 (7)	.081	110 dB	-0.028 (7)	.978
	120 dB	-1.616 (7)	.150	120 dB	-0.710 (7)	.501
	Average %PPI	-1.078 (7)	.317	Average %PPI	-2.863 (7)	.024
	HP	-2.611 (7)	.035	HP	-2.318 (7)	.054
OFS	BW	-42.291 (7)	.000	BW	-23.003 (7)	.000
	bland food	10.358 (7)	.000	bland food	-5.206 (7)	.001
	HCA	-1.271 (7)	.244	HCA	1.247 (7)	.252
	HZ	-1.151 (7)	.288	HZ	-0.125 (7)	.904
	VA	-3.698 (7)	.008	VA	-1.263 (7)	.247
	CD	-2.151 (7)	.069	CD	-0.732 (7)	.488
	CT	-4.546 (7)	.003	CT	-0.286 (7)	.783
	110 dB	0.253 (7)	.808	110 dB	1.834 (7)	.109
	120 dB	-1.306 (7)	.233	120 dB	-1.078 (7)	.317
	Average %PPI	-1.053 (7)	.327	Average %PPI	1.099 (7)	.308
	HP	-0.621 (7)	.555	HP	1.152 (7)	.287

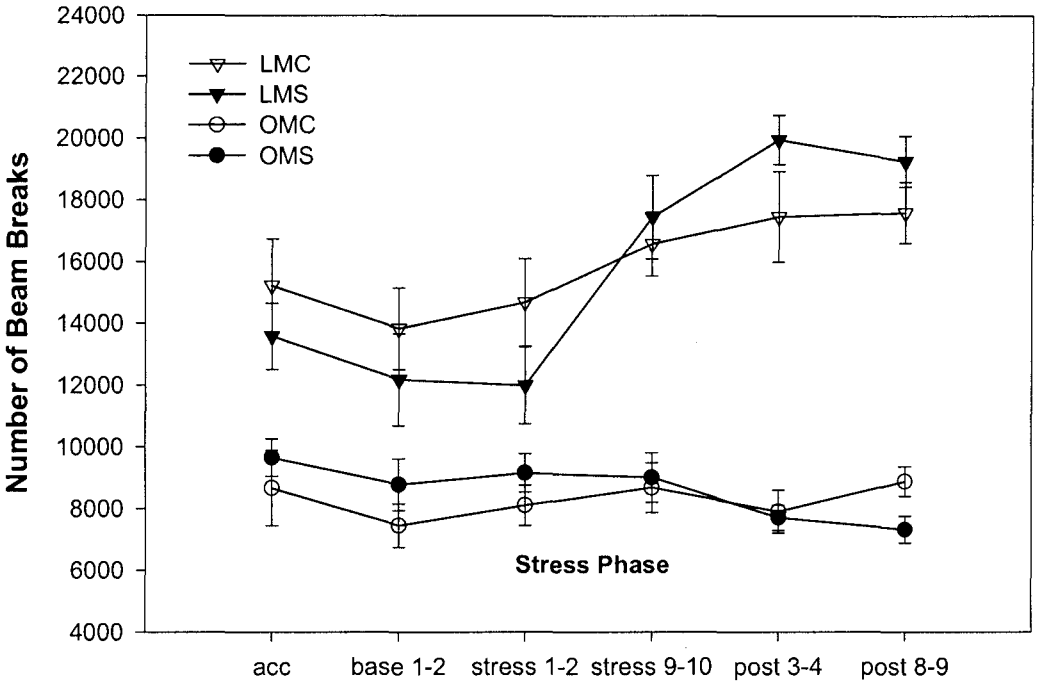

Figure 43. Horizontal activity for males during each testing session.

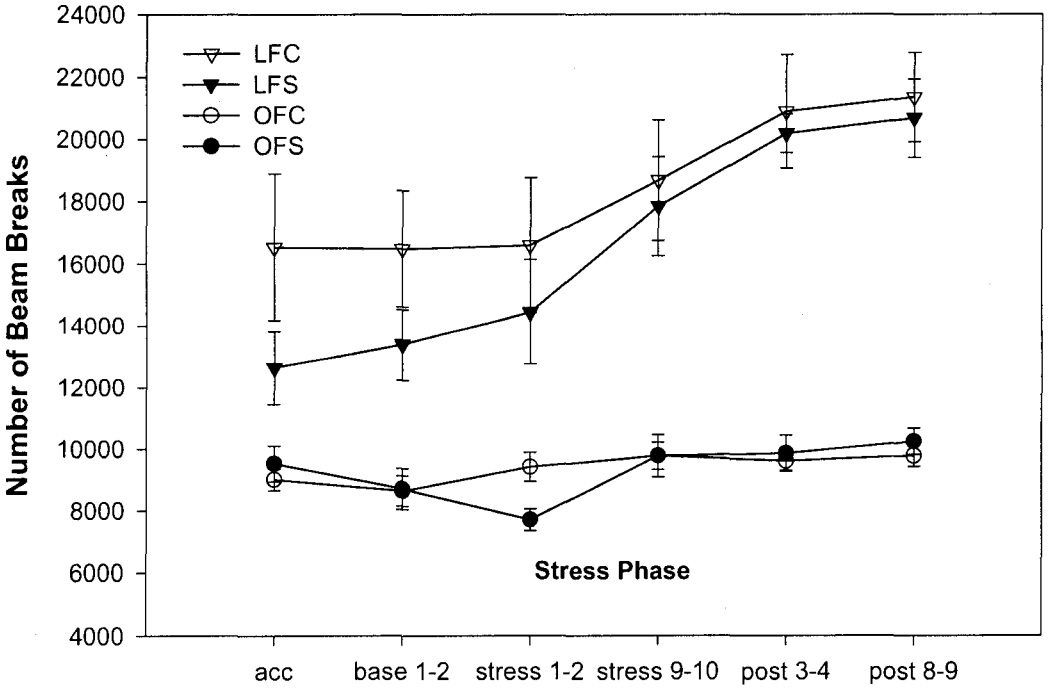

Figure 44. Horizontal activity for females during each testing session.

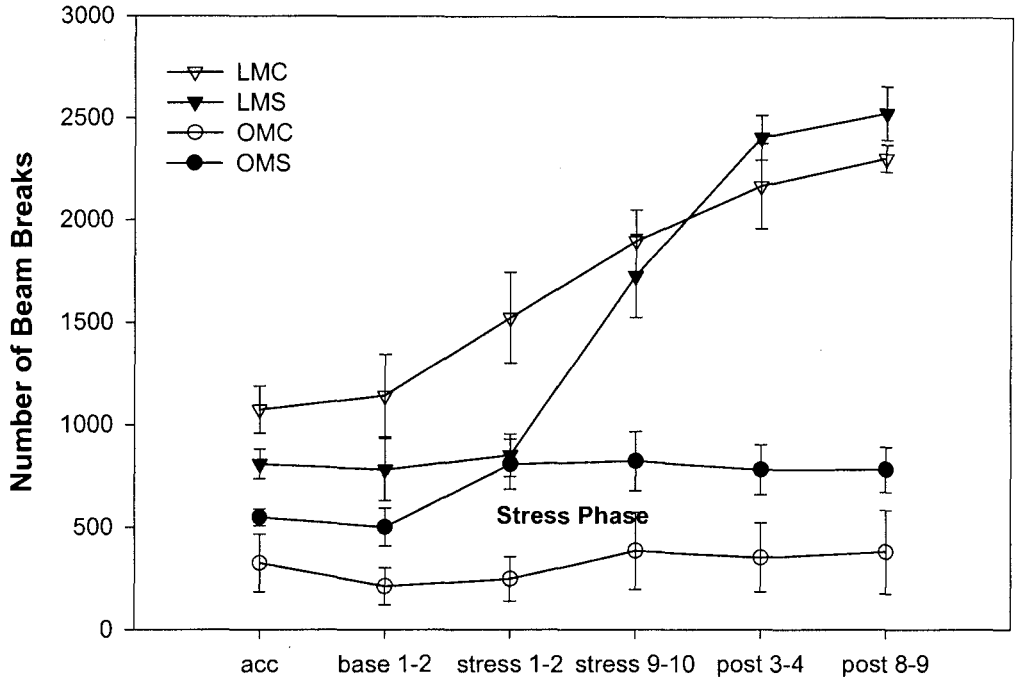

Figure 45. Vertical activity for males during each testing session.

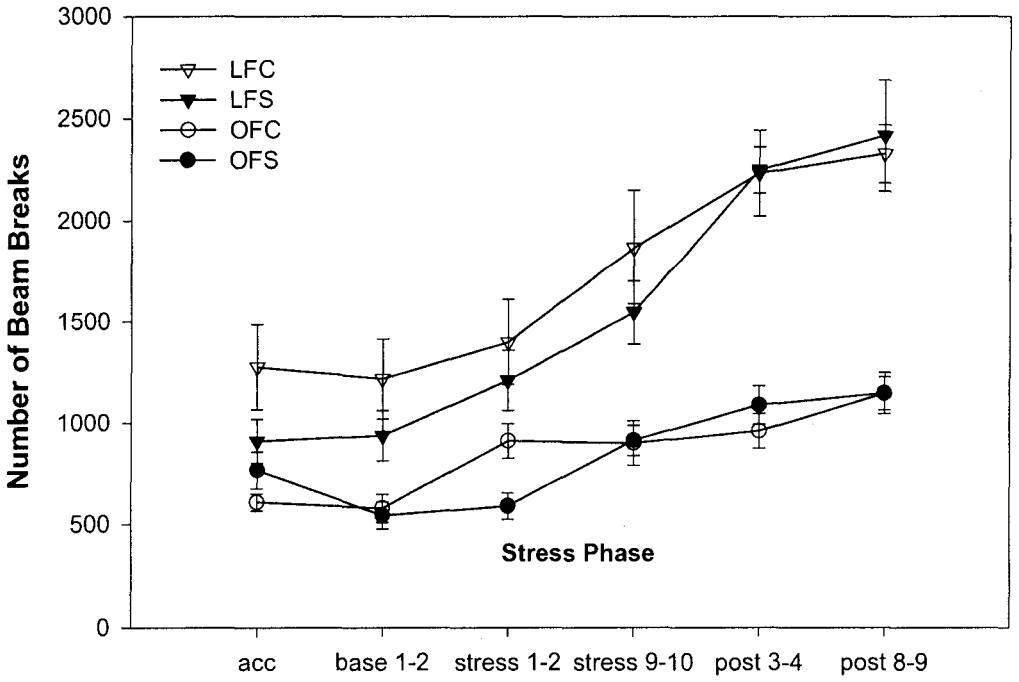

Figure 46. Vertical activity for females during each testing session.

APPENDIX B: REFERENCES

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